

APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: 33410, A NOVEL HUMAN CARBOXYLESTERASE  
FAMILY MEMBER AND USES THEREOF

APPLICANT: RORY A. J. CURTIS

FOR DEPOSIT

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL298424979US

I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

Date of Deposit August 21, 2001

Signature

*Samantha Bell*

Samantha Bell  
Typed or Printed Name of Person Signing Certificate

# 33410, A NOVEL HUMAN CARBOXYLESTERASE FAMILY MEMBER AND USES THEREOF

## Related Applications

5        This application claims priority to U.S. provisional application number 60/226,774  
filed on August 21, 2000, the contents of which are incorporated herein by reference.

## Background of the Invention

Higher eukaryotes have many distinct carboxylesterases. Among the different types  
of carboxylesterases are those that act on carboxylic esters. Carboxylesterases have been  
10    classified into three categories (A, B and C) on the basis of differential patters of inhibition  
by organophosphates (Myers, M. et al. (1988) *Mol. Biol. Evol.* 5(2):113-119). The sequence  
of a number of type-B carboxylesterase indicates that the majority are evolutionarily related.  
Members of the type B carboxylesterase include acetylcholinesterases from  
vertebrates and drosophila, mammalian cholinesterases, mammalian bile salt  
15    activated lipases, among others.

Neuroligins are cell surface molecules composed of five domains: an N-terminal  
cleaved signal sequence, a large extracellular domain homologous to carboxylesterases, a  
linker domain between the transmembrane region and the carboxylesterase homology  
domain, a single transmembrane region, and a cytoplasmic tail (Ichtchenko, K. et al. (1996)  
20    *J. Biol. Chem.* 271(5):2676-2682). Sequence comparisons place the neuroligins in the large  
family of carboxylesterase homology domain proteins that includes thyroglobulin,  
acetylcholinesterase, and gliotactin. However, neuroligins are only distantly related  
to these proteins, and thus appear to form a unique subset of the carboxylesterase family. At  
least three neuroligins have been cloned, namely Neuroligins 1, 2 and 3 (Ichtchenko, K. et  
25    al. (1995) *Cell* 81:435-443; Ichtchenko, K. et al. (1996) *supra*). These three neuroligins are  
expressed at high levels in the brain, primarily in neurons. Neuroligins have been shown to  
mediate cell adhesion events associated with neuronal development and/or maintenance.  
For example, Neuroigin 1 has been found to be enriched in postsynaptic densities where it  
may recruit receptors, channels, and signal transduction molecules at synaptic sites of cell  
30    adhesion (Song et al. (1999) *Proc. Natl. Acad. Sci.* 96(3):1100-5).

Functionally, neuroligins bind tightly, in a calcium-dependent manner, to the  
extracellular domains of the polymorphic cell surface proteins known as  $\beta$ -neurexins.  
Neurexins are neuronal cell surface proteins that exhibit a high degree of diversity

T0139-00466

(Ushkaryov et al. (1994) *J. Biol. Chem.* 269: 11987-11992). Neuroligin- $\beta$ -neurexin interactions have been implicated in mediating recognition processes between neurons that give rise to neuronal developmental events such as synaptogenesis (e.g., specification of excitatory synapses) (Brose, N. (1999) *Naturwissenschaften* 86(11):516-24).

5

### Summary of the Invention

The present invention is based, in part, on the discovery of a novel carboxylesterase family member, referred to herein as "33410". The nucleotide sequence of a cDNA encoding 33410 is shown in SEQ ID NO:1, and the amino acid sequence of a 33410 polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequences of the coding region are depicted in SEQ ID NO:3.

Accordingly, in one aspect, the invention features a nucleic acid molecule that encodes a 33410 protein or polypeptide, e.g., a biologically active portion of the 33410 protein. In a preferred embodiment the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated 33410 nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_\_. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_\_. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_\_, wherein the nucleic acid encodes a full length 33410 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs that include a 33410 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 33410 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing 33410 nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 33410-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a 33410 encoding nucleic acid molecule are provided.

5 In another aspect, the invention features 33410 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of 33410-mediated or -related disorders, e.g., a cell adhesion disorder; a disorder involving aberrant cellular proliferation or differentiation (e.g., a cancer); a CNS disorder, such as a neurodegenerative disorder, *e.g.*, Alzheimer's disease, 10 dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, Jakob-Creutzfeldt disease, AIDS related dementia, familial infantile convulsions, paroxysmal choreoathetosis; a psychiatric disorder (*e.g.*, depression, schizophrenic disorders, korsakoff's psychosis, mania, anxiety disorders, or phobic 15 disorders); a learning or memory disorder (*e.g.*, amnesia or age-related memory loss; and migraine).

In another embodiment, the invention provides 33410 polypeptides having a 33410 activity. Preferred polypeptides are 33410 proteins including at least one carboxylesterase domain, a signal peptide and at least one transmembrane domain, and, preferably, having a 20 33410 activity, *e.g.*, a 33410 activity as described herein (*e.g.*, the modulation of one or more of: a cell-cell (*e.g.*, neuron-neuron, or neuron-glia) recognition event or adhesion; synaptogenesis; membrane excitability; neurite outgrowth; signal transduction; or cell (*e.g.*, neural or a cancer cell) proliferation, growth, differentiation, or migration).

In other embodiments, the invention provides 33410 polypeptides, *e.g.*, a 33410 25 polypeptide having the amino acid sequence shown in SEQ ID NO:2; the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization 30 conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_, wherein the nucleic acid encodes a full length 33410 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs that include a 33410 nucleic acid molecule described herein.

In a related aspect, the invention provides 33410 polypeptides or fragments operatively linked to non- 33410 polypeptides to form fusion proteins.

5 In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 33410 polypeptides.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 33410 polypeptides or nucleic acids.

10 In yet another aspect, the invention features a method of evaluating, or identifying, an agent, e.g., an agent as described herein, e.g., a compound (e.g., a polypeptide, peptide, a peptide fragment, a peptidomimetic, a small molecule), for the ability to modulate, e.g. inhibit, the activity or expression of a 33410 polypeptide. Such agents are useful for treating or preventing cardiovascular disorders (e.g., an endothelial cell disorder) or proliferation-related disorders, e.g., cancer, as described herein. The method includes:

15 providing a test agent, and a 33410 nucleic acid or polypeptide, or a cell expressing an 33410 (e.g., a cancer cell or cell line);

contacting said test agent, and said 33410 nucleic acid or polypeptide, or said cell expressing said 33410, under conditions that allow an interaction (e.g., activity or expression) between said 33410 nucleic acid or polypeptide and said test agent to occur; and

20 determining whether said test agent interacts with, e.g., binds the 33410 nucleic acid or polypeptide, or modulates, e.g., inhibits, the expression or activity of said 33410 polypeptide. E.g. wherein interacting with or binding the 33410 nucleic acid or polypeptide, or wherein a change, e.g., a decrease, in the level of activity or expression between said 33410 polypeptide in the presence of the test agent relative to the activity or expression in the absence  
25 of the test agent, is indicative of modulation, e.g., inhibition, of modulation of 33410 activity or expression.

In a preferred embodiment, the method further comprises the step of evaluating the test agent in the 33410-expressing cell, e.g., an endothelial or a cancer cell, *in vitro*, or *in vivo* (e.g., in a subject, e.g., a patient having a cancer or a cardiovascular disorder), to thereby determine  
30 the effect of the test agent in the activity or expression of the 33410.

In a preferred embodiment, the contacting step occurs *in vitro* or *ex vivo*. For example, a sample, e.g., a blood, biopsy or tissue sample, is obtained from the subject. Preferably, the sample contains a 33410-expressing cell.

093433-093401

In a preferred embodiment, the contacting step occurs *in vivo*. For example, by administering to the subject a detectably labeled agent that interacts with the 33410 nucleic acid or polypeptide, such that a signal is generated relative to the level of activity or expression of the 33410 nucleic acid or polypeptide.

5 In a preferred embodiment, the test agent is an inhibitor (partial or complete inhibitor) of the 33410 polypeptide activity or expression.

In preferred embodiments, the test agent is a peptide, a small molecule, e.g., a member of a combinatorial library (e.g., a peptide or organic combinatorial library, or a natural product library), or an antibody, or any combination thereof.

10 In additional preferred embodiments, the test agent is an antisense, a ribozyme, a triple helix molecule, or any combination thereof.

In a preferred embodiment, a plurality of test agents, e.g., library members, is tested. In a preferred embodiment, the plurality of test agents, e.g., library members, includes at least 10,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ , or  $10^8$  compounds. In a preferred embodiment, the  
15 plurality of test agents, e.g., library members, share a structural or functional characteristic.

In a preferred embodiment, test agent is a peptide or a small organic molecule.

In a preferred embodiment, the method is performed in cell-free conditions (e.g., a reconstituted system).

In a preferred embodiment, the method further includes: contacting said agent with a  
20 test cell, or a test animal, to evaluate the effect of the test agent on the activity or expression of 33410.

In a preferred embodiment, the ability of the agent to modulate the activity or expression of 33410 is evaluated in a second system, e.g., a cell-free, cell-based, or an animal system.

25 In a preferred embodiment, the ability of the agent to modulate the activity or expression of 33410 is evaluated in a cell based system, e.g., a two-hybrid assay.

In still another aspect, the invention provides a process for modulating 33410 polypeptide or nucleic acid expression or activity, e.g. using one or more of the screened compounds. In certain embodiments, the methods involve treatment of conditions related to  
30 aberrant activity or expression of the 33410 polypeptides or nucleic acids, such as neurological conditions. For example, one or more of the screened compounds can be used to modulate one or more of cell-cell adhesion events, membrane excitability, neurite outgrowth, synaptogenesis, signal transduction, cell (e.g., neural cell) proliferation, growth,

5 In yet another aspect, the invention provides methods for inhibiting the proliferation or inducing the killing, of a 33410-expressing cell, e.g., a 33410-expressing hyperproliferative cell, comprising contacting the cell with a compound (e.g., a compound identified using the methods described herein) that modulates the activity, or expression, of the 33410 polypeptide or nucleic acid.

In a preferred embodiment, the 33410-expressing cell is found in a solid tumor, a soft tissue tumor, or a metastatic lesion. Preferably, the tumor is a sarcoma, a carcinoma, or an adenocarcinoma. Preferably, the cell is found in a cancerous or pre-cancerous tissue, e.g., a cancerous or pre-cancerous tissue where a 33410 polypeptide or nucleic acid is expressed.

20 In a preferred embodiment, the compound is an inhibitor of a 33410 polypeptide. Preferably, the inhibitor is chosen from a peptide, a phosphopeptide, a peptidomimetic, e.g., a phosphonate analog of a peptide substrate, a small organic molecule, a small inorganic molecule and an antibody (e.g., an antibody conjugated to a therapeutic moiety selected from a cytotoxin, a cytotoxic agent and a radioactive metal ion).

In a preferred embodiment, the compound is administered in combination with a cytotoxic agent. Examples of cytotoxic agents include anti-microtubule agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, an anti-metabolite, a mitotic inhibitor, an alkylating agent, an intercalating agent, an agent capable of interfering with a signal transduction pathway, an agent that promotes apoptosis or necrosis, and radiation.

- 6 -

in a subject. Preferably, the method includes comprising administering to the subject (e.g., a mammal, e.g., a human) an effective amount of a compound (e.g., a compound identified using the methods described herein) that modulates the activity, or expression, of the 33410 polypeptide or nucleic acid.

5 In a preferred embodiment, the disorder is a cancerous or pre-cancerous condition. Most preferably, the disorder is a cancer, e.g., a solid tumor, a soft tissue tumor, or a metastatic lesion. Preferably, the cancer is a sarcoma, a carcinoma, or an adenocarcinoma. Preferably, the cancer is found in a tissue where a 33410 polypeptide or nucleic acid is expressed, e.g., breast, ovarian, colon, liver, lung, kidney, or brain cancer. Most preferably,  
10 the cancer is found in the breast, ovary, colon, liver and lung.

In a preferred embodiment, the disorder is an endothelial cell disorder; is a disorder characterized by aberrant, unregulated, or unwanted endothelial cell activity, e.g., proliferation, migration, angiogenesis, or vascularization; or aberrant expression of cell surface adhesion molecules or genes associated with angiogenesis. Examples of endothelial  
15 cell disorders include tumorigenesis, tumor metastasis, psoriasis, diabetic retinopathy, endometriosis, Grave's disease, ischemic disease (e.g., atherosclerosis), and chronic inflammatory diseases (e.g., rheumatoid arthritis).

In a preferred embodiment, the compound is an inhibitor of a 33410 polypeptide. Preferably, the inhibitor is chosen from a peptide, a phosphopeptide, a small organic  
20 molecule, a small inorganic molecule and an antibody (e.g., an antibody conjugated to a therapeutic moiety selected from a cytotoxin, a cytotoxic agent and a radioactive metal ion). The inhibitor can also be a trypsin inhibitor or a derivative thereof, or a peptidomimetic, e.g., a phosphonate analog of a peptide substrate.

In a preferred embodiment, the compound is an inhibitor of a 33410 nucleic acid,  
25 e.g., an antisense, a ribozyme, or a triple helix molecule.

In a preferred embodiment, the compound is administered in combination with a cytotoxic agent. Examples of cytotoxic agents include anti-microtubule agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, an anti-metabolite, a mitotic inhibitor, an alkylating agent, an intercalating agent, an agent capable of interfering with a  
30 signal transduction pathway, an agent that promotes apoptosis or necrosis, and radiation.

In a preferred embodiment, the subject is a mammal, e.g., a human; a patient, e.g., a patient with a cancer or a cardiovascular condition.



The invention also provides assays for determining the activity of or the presence or absence of 33410 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis. Preferably, the biological sample includes a cancerous or pre-cancerous cell or tissue. For example, the cancerous tissue can be a solid tumor, a soft tissue tumor, or a metastatic lesion. Preferably, the cancerous tissue is a sarcoma, a carcinoma, or an adenocarcinoma. In other embodiments, the biological sample includes endothelial cells.

In further aspect the invention provides assays for determining the presence or absence of a genetic alteration in a 33410 polypeptide or nucleic acid molecule, including for disease diagnosis. Preferably, the biological sample includes a cancerous or pre-cancerous cell or tissue. For example, the cancerous tissue can be a solid tumor, a soft tissue tumor, or a metastatic lesion. Preferably, the cancerous tissue is a sarcoma, a carcinoma, or an adenocarcinoma. In other embodiments, the biological sample includes endothelial cells.

In another aspect, the invention features a method of diagnosing, or staging, a 33410-mediated disorder, e.g., a neurological disorder, or a cancer disorder, in a subject. The method includes evaluating the expression or activity of a 33410 nucleic acid or polypeptide, thereby diagnosis or staging the disorder. In a preferred embodiment, the expression or activity is compared with a reference value, e.g., a difference in the expression or activity level of the 33410 nucleic or polypeptide relative to a reference, e.g., a normal subject or a cohort of normal subjects is indicative of the disorder, or a stage in the disorder.

In a preferred embodiment, the subject is a human. For example, the subject is a human suffering from, or at risk of, a cardiovascular or a cancer disorder as described herein.

In a preferred embodiment, the evaluating step occurs *in vitro* or *ex vivo*. For example, a sample, e.g., a blood or tissue sample, a biopsy, is obtained from the subject.

Preferably, the sample contains a cancer or an endothelial cell.

In a preferred embodiment, the evaluating step occurs *in vivo*. For example, by administering to the subject a detectably labeled agent that interacts with the 33410-associated nucleic acid or polypeptide, such that a signal is generated relative to the level of activity or expression of the 33410 nucleic acid or polypeptide.

In preferred embodiments, the method is performed: on a sample from a subject, a sample from a human subject; e.g., a sample of a patient suffering from, or at risk of, a cardiovascular or a cancer disorder as described herein; to determine if the individual from which the target nucleic acid or protein is taken should receive a drug or other treatment; to

diagnose an individual for a disorder or for predisposition to resistance to treatment, to stage a disease or disorder.

In a still further aspect, the invention provides methods for evaluating the efficacy of a treatment of a disorder, e.g., proliferative disorder, e.g., a cancer (e.g., breast, ovarian, colon, liver or lung cancer); or an endothelial cell disorder. The method includes: treating a  
 5 subject, e.g., a patient or an animal, with a protocol (e.g., treating a subject with one or more of: chemotherapy, radiation, and/or a compound identified using the methods described herein); and evaluating the expression of a 33410 nucleic acid or polypeptide before and after treatment. A change, e.g., a decrease or increase, in the level of a 33410 nucleic acid  
 10 (e.g., mRNA) or polypeptide after treatment, relative to the level of expression before treatment, is indicative of the efficacy of the treatment of the disorder.

In a preferred embodiment, the disorder is a cancer of the breast, ovary, colon, lung, or liver. In other embodiments, the disorder is an endothelial cell disorder. The level of 33410 nucleic acid or polypeptide expression can be detected by any method described  
 15 herein.

In a preferred embodiment, the evaluating step includes obtaining a sample (e.g., a tissue sample, e.g., a biopsy, or a fluid sample) from the subject, before and after treatment and comparing the level of expressing of a 33410 nucleic acid (e.g., mRNA) or polypeptide before and after treatment.

In another aspect, the invention provides methods for evaluating the efficacy of a  
 20 therapeutic or prophylactic agent (e.g., an anti-neoplastic agent). The method includes: contacting a sample with an agent (e.g., a compound identified using the methods described herein, a cytotoxic agent) and, evaluating the expression or activity of a 33410 nucleic acid or polypeptide in the sample before and after the contacting step. A change, e.g., a decrease  
 25 or increase, in the level of 33410 nucleic acid (e.g., mRNA) or polypeptide in the sample obtained after the contacting step, relative to the level of expression in the sample before the contacting step, is indicative of the efficacy of the agent. The level of 33410 nucleic acid or polypeptide expression can be detected by any method described herein.

In a preferred embodiment, the sample includes cells obtained from a cancerous  
 30 tissue where a 33410 polypeptide or nucleic acid is obtained, e.g., a cancer of the breast, ovary, colon, lung, or liver.

In a preferred embodiment, the sample is a tissue sample (e.g., a biopsy), a bodily fluid, cultured cells (e.g., a tumor cell line).

In a preferred embodiment, the sample includes endothelial cells.

In another aspect, the invention features a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 33410 molecule. In one embodiment, the capture probe is a nucleic acid, e.g., a probe complementary to a 33410 nucleic acid sequence. In another embodiment, the capture probe is a polypeptide, e.g., an antibody specific for 33410 polypeptides. Also featured is a method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

#### Brief Description of the Drawings

*Figure 1* depicts a hydropathy plot of human 33410. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of human 33410 are indicated. Polypeptides of the invention include 33410 fragments which include: all or part of a hydrophobic sequence, i.e., a sequence above the dashed line, e.g., the sequence from about amino acid 60 to 72, from about 260 to 277, and from about 780 to 793; all or part of a hydrophilic sequence, i.e., a sequence below the dashed line, e.g., the sequence of from about amino acid 330 to 350, from about 480 to 505, and from about 695 to 720; and/or a sequence which includes a cysteine, or a glycosylation site.

*Figure 2* depicts an alignment of the carboxylesterase domain of human 33410 with a consensus amino acid sequence derived from a hidden Markov model (HMM). The upper sequence is the consensus amino acid sequence (SEQ ID NO:4), while the lower amino acid sequence corresponds to amino acids 42 to 601 of SEQ ID NO:2.

*Figure 3* depicts alignment of the rat neuroligin-2 amino acid sequence and the human 33410 (SEQ ID NO:2) amino acid sequences. The location of the transmembrane domain in the rat neuroligin-2 and 33410 amino acid sequences is indicated as "TM1".

*Figure 4* depicts alignment of the partial human KIAA1366 (Genbank Accession Number AB037787 and the human 33410 amino acid sequences (SEQ ID NO:2).

### Detailed Description

The human 33410 sequence (Figure 1; SEQ ID NO:1), which is approximately 4667 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 2508 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence encodes a 835 amino acid protein (SEQ ID NO:2). The human 33410 protein of SEQ ID NO:2 and Figure 2 includes an amino-terminal hydrophobic amino acid sequence, consistent with a signal sequence, of about 14 amino acids (from amino acid 1 to about amino acid 14 of SEQ ID NO:2), which upon cleavage results in the production of a mature protein form.

Human 33410 contains the following regions or other structural features: a carboxylesterase domain (PFAM Accession Number PF00135) located at about amino acid residues 42 to 601 of SEQ ID NO:2 which includes a carboxylesterase type-B signature 2 domain (Prosite Accession Number PS00941) at about amino acid 139 to 149 of SEQ ID NO:2; and one predicted transmembrane domain at about amino acid 676 to 698 of SEQ ID NO:2.

The 33410 protein also includes the following domains: four predicted N-glycosylation sites (PS00001) at about amino acids 98 to 101, 136 to 139, 522 to 525, and 823 to 826 of SEQ ID NO:2; three predicted glycosaminoglycan attachment sites (PS00002) at about amino acids 264 to 267, 718 to 721 and 793 to 796 of SEQ ID NO:2; three predicted cAMP- and cGMP-dependent protein kinase phosphorylation sites (PS00004) at about amino acids 331 to 334, 447 to 450, and 710 to 713 of SEQ ID NO:2; six predicted Protein Kinase C phosphorylation sites (PS00005) at about amino acids 156 to 158, 430 to 432, 467 to 469, 619 to 621, 640 to 642, and 832 to 834 of SEQ ID NO:2; five predicted Casein Kinase II phosphorylation sites (PS00006) located at about amino 35 to 38, 187 to 190, 223 to 226, 404 to 407, and 524 to 527 of SEQ ID NO:2; one predicted Tyrosine kinase phosphorylation site (PS00007) at about amino acids 664 to 672 of SEQ ID NO:2; fourteen predicted N-myristoylation sites (PS00008) from about amino 10 to 15, 18 to 23, 30 to 35, 71 to 76, 95 to 100, 112 to 117, 198 to 203, 263 to 268, 292 to 297, 381 to 386, 400 to 405, 636 to 641, 716 to 721, and 750 to 755 of SEQ ID NO:2; one predicted amidation site (PS00009) at about amino acid 174 to 177 of SEQ ID NO:2; and one predicted prokaryotic membrane lipoprotein lipid attachment site (PS00013) at about amino acid 260 to 270 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer et al. (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

A plasmid containing the nucleotide sequence encoding human 33410 (clone Fbh33410FL) was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 33410 protein contains a significant number of structural characteristics in common with members of the carboxylesterase family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics. Carboxylesterase family members are known to act on carboxylic esters, such as acetylcholinesterase. Based on the differential patterns of inhibition by organophosphates, carboxylesterases have been classified into three categories (A, B and C) (Myers, M. et al. (1988) *Mol. Biol. Evol.* 5(2):113-119). 33410 proteins of the invention include a carboxylesterase type B signature 2 domain located at about amino acids 139 to 149 of SEQ ID NO:2, which suggests that the 33410 proteins belong to the carboxylesterase type B family.

Carboxylesterase family members are characterized by a catalytic triad of amino acids: a serine, a glutamate or aspartate and a histidine. The sequence around the active site serine is well conserved and can be used as a signature pattern. A second signature pattern is located in the N-terminal section and contains a cysteine involved in disulfide bond formation. Typical consensus patterns of carboxylesterases are F-[GR]-G-x(4)-[LIVM]-x-[LIV]-x-G-x-S-[STAG]-G (where S is the active site residue) and [ED]-D-C-L-[YT]-[LIV]-[DNS]-[LIV]-[LIVFYW]-x-[PQR] (where C is involved in a disulfide bond). 33410

proteins have a similar pattern starting at about amino acid 252 to 267 of SEQ ID NO:2 as follows: FGGDPERITIFGSGAG.

33410 proteins of the invention are homologous to rat neuroligin proteins, in particular, the rat neuroligin-2 protein (Figure 4). Thus, the proteins of the invention are members of the neuroligin subfamily of carboxylesterase type B proteins. As used herein, the term “neuroligin” refers to cell surface molecules composed of five domains: an N-terminal cleaved signal sequence, a large extracellular domain homologous to esterases, a linker domain between the transmembrane region and the esterase homology domain, a single transmembrane region, and a cytoplasmic tail (Ichtkchenko, K. et al. (1996) *J. Biol. Chem.* 271(5):2676-2682). Neurexin binding is known to be calcium-dependent and is mediated by an EF hand motif. 33410 contain several structural features of neuroligin family members. For example, 33410 proteins have an extracellular domain located at about amino acids 1-675 of SEQ ID NO:2 (which includes a carboxylesterase domain located at amino acids 42 to 601 of SEQ ID NO:2), a transmembrane domain located at about amino acids 676-698, and a short cytoplasmic domain located at about amino acids 699-835 of SEQ ID NO:2. 33410 proteins further include an EF hand motif from about amino acid 387 to 416 of SEQ ID NO:2, and six conserved cysteine residues located at about amino acids 106, 141, 270, 317, 328, 487 and 521 of SEQ ID NO:2.

Typically, members of the neuroligins are expressed at high levels in the brain, primarily in neurons. Typically, neuroligins are capable of mediating cell adhesion events associated with development and/or maintenance, e.g., neural events such as synaptogenesis, recruitment of receptors, channels, and signal transduction molecules at synaptic sites (e.g., at excitatory synapses) (Song et al. (1999) *Proc. Natl. Acad. Sci.* 96(3):1100-5. Typically, neuroligins are capable of interacting with a cell surface protein, e.g., a neurexin (e.g., a  $\beta$ -neurexins). Typically, neuroligin- $\beta$ -neurexin interactions mediate cell adhesion events, e.g., neuron-neuron, or neuron-glia cell adhesion events.

A 33410 polypeptide can include a “carboxylesterase domain” or regions homologous with an “carboxylesterase” domain. A 33410 can optionally further include at least one transmembrane domain, at least one extracellular domain, and at least one intracellular domain. A 33410 can optionally further include at least one, two, three, preferably four N-glycosylation sites; at least one, two preferably three cAMP/cGMP phosphorylation sites; at least one, two, three, four, five, preferably six protein kinase C sites; at least one, two, three, four, preferably five casein kinase II sites; at least one, two,

three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, and preferably fourteen N-myristoylation sites; at least one tyrosine phosphorylation site, at least one amidation site; and at least one, two and preferably three glycosaminoglycan attachment site.

As used herein, the term “carboxylesterase domain” refers to a protein domain which is includes a carboxylesterase type B signature 2 domain. Preferably, the carboxylesterase type B signature 2 domain is about 5 to 20 amino acids, more preferably 8-15, most preferably 11 amino acids and includes the sequence [EDX(0,1)CLYX]. Most preferably, the carboxylesterase type B signature 2 domain has the amino acid sequence: EDCLYNIYVP located at about amino acids 139 to 149 of SEQ ID NO:2. Preferably, the carboxylesterase domain has an amino acid sequence of about 450 to about 650 amino acid residues and having a bit score for the alignment of the sequence to the carboxylesterase domain (HMM) of at least 100. Preferably, a carboxylesterase domain includes at least about 450 to about 600 amino acids, more preferably about 500 to about 575 amino acid residues, about 550 to 570, or about 559 amino acids and has a bit score for the alignment of the sequence to the carboxylesterase domain (HMM) of at least 200, preferably 300, more preferably 400 or greater. The carboxylesterase domain (HMM) has been assigned the PFAM Accession (PF00135) (<http://genome.wustl.edu/Pfam/html>). An alignment of the carboxylesterase domain (from about amino acids 42 to about 601 of SEQ ID NO:2) of human 33410 with a consensus amino acid sequence derived from a hidden Markov model (PFAM) is depicted in Figure 3.

In a preferred embodiment, 33410 polypeptide or protein has a “carboxylesterase domain” or a region which includes at least about 400 to about 650 amino acids, preferably 450 to about 600 amino acids, more preferably about 500 to about 570 amino acid residues, about 550 to 570, or about 559 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a “carboxylesterase domain,” e.g., the carboxylesterase domain of human 33410 (e.g., residues 42 to 601 of SEQ ID NO:2).

To identify the presence of an “carboxylesterase” domain in a 33410 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters ([http://www.sanger.ac.uk/Software/Pfam/HMM\\_search](http://www.sanger.ac.uk/Software/Pfam/HMM_search)). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for

determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of an "carboxylesterase domain" domain in the amino acid sequence of human 33410 at about residues 42 to 601 of SEQ ID NO:2 (see Figure 1).

In one embodiment, a 33410 protein includes at least one transmembrane domain. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length that spans a phospholipid membrane. More preferably, a transmembrane domain includes about at least 16, 18, 20, 21, 23, 25, 30, 35 or 40 amino acid residues and spans a phospholipid membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an  $\alpha$ -helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, e.g., leucines, isoleucines, tyrosines, or tryptophans.

Transmembrane domains are described in, for example, <http://pfam.wustl.edu/cgi-bin/getdesc?name=7tm-1>, and Zagotta W.N. et al, (1996) *Annual Rev. Neurosci.* 19: 235-63, the contents of which are incorporated herein by reference.

In a preferred embodiment, 33410 polypeptide or protein has a "transmembrane domain" or a region which includes at least about 1 to 45, more preferably about 10 to 35, even more preferably about 20 to 25 or 23 amino acid residues and has at least about 50%, 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "transmembrane domain" e.g., the transmembrane domain of human 33410 (e.g., residues 676 to 698 of SEQ ID NO:2).

A 33410 protein further includes a predicted N-terminal extracellular domain located at about amino acids 1-675 (or 15-675 of the mature protein) of SEQ ID NO:2. As used herein, an "N-terminal extracellular domain" includes an amino acid sequence about 1-800, preferably about 200-700, and even more preferably about 300-680 or 675, amino acid residues in length and is located outside of a cell or extracellularly. The C-terminal amino acid residue of a "N-terminal extracellular domain" is adjacent to an N-terminal amino acid residue of a transmembrane domain in a naturally occurring 33410 or 33410-like protein.



For example, an N-terminal cytoplasmic domain is located at about amino acid residues 1-675 of SEQ ID NO:2.

In a preferred embodiment 33410 polypeptide or protein has an “N-terminal extracellular domain” or a region which includes at least about 1-800, preferably about 200-700, and even more preferably about 300-680 or 675 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with an “N-terminal extracellular domain,” e.g., the N-terminal extracellular domain of human 33410 (e.g., residues 1-675 of SEQ ID NO:2). Preferably, the N-terminal extracellular domain is capable of interacting (e.g., binding to) with an extracellular signal (e.g., a neurexin) and/or modulating cell adhesion.

In another embodiment, a 33410 protein includes a "C-terminal cytoplasmic domain", also referred to herein as a C-terminal cytoplasmic tail, in the sequence of the protein. As used herein, a "C-terminal cytoplasmic domain" includes an amino acid sequence having a length of at least about 50 to 200, preferably 100 to 150, and more preferably 136 amino acid residues and is located within a cell or within the cytoplasm of a cell. Accordingly, the N-terminal amino acid residue of a "C-terminal cytoplasmic domain" is adjacent to a C-terminal amino acid residue of a transmembrane domain in a naturally-occurring 33410 or 33410-like protein. For example, a C-terminal cytoplasmic domain is found at about amino acid residues 699-835 of SEQ ID NO:2.

20 In a preferred embodiment, a 33410 polypeptide or protein has a C-terminal cytoplasmic domain or a region which includes at least about 50 to 200, preferably 100 to 150, and more preferably 136 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with an "C-terminal cytoplasmic domain," e.g., the C-terminal cytoplasmic domain of human 33410 (e.g., residues 699-835 of SEQ ID NO:2).

25           A 33410 molecule can further include a signal sequence. As used herein, a "signal sequence" refers to a peptide of about 10-40 amino acid residues in length which occurs at the N-terminus of secretory and integral membrane proteins and which contains a majority of hydrophobic amino acid residues. For example, a signal sequence contains at least about 12-30 amino acid residues, preferably about 13-20 amino acid residues, more preferably  
30   about 14 amino acid residues, and has at least about 40-70%, preferably about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (e.g., alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a protein

containing such a sequence to a lipid bilayer. For example, in one embodiment, a 33410 protein contains a signal sequence of about amino acids 1-14 of SEQ ID NO:2. The "signal sequence" is cleaved during processing of the mature protein. The mature 33410 protein corresponds to amino acids 15 to 835 of SEQ ID NO:2.

5 As the 33410 polypeptides of the invention may modulate 33410-mediated activities, they may be useful as or for developing novel diagnostic and therapeutic agents for 33410-mediated or related disorders, as described below.

As used herein, a "33410 activity", "biological activity of 33410" or "functional activity of 33410", refers to an activity exerted by a 33410 protein, polypeptide or nucleic acid molecule on e.g., a 33410-responsive cell or on a 33410 substrate, e.g., a protein  
10 substrate, as determined *in vivo* or *in vitro*. In one embodiment, a 33410 activity is a direct activity, such as an association with a 33410 target molecule. A "target molecule" or "binding partner" is a molecule with which a 33410 protein binds or interacts in nature, e.g., a cell surface molecule, e.g., a neuroligin. A 33410 activity can also be an indirect activity,  
15 e.g., a cellular signaling activity mediated by interaction of the 33410 protein with a 33410 receptor. For example, the 33410 proteins of the present invention can have one or more of the following activities: 1) ability to catalyze the hydrolysis of carboxylic esters; (2) ability to mediate cell-cell (e.g., neuron-neuron, or neuron-glia) recognition events, adhesion or attachment (3) ability to interact with a cell surface protein (e.g., neuroligin) or an  
20 extracellular component; (4) ability to modulate cell migration, (5) ability to modulate patterning, (6) ability to modulate proliferation, and/or differentiation, of a cell (e.g., a neural or a cancer cell); (7) ability to modulate embryonic development and differentiation; (8) ability to modulate morphogenesis; (9) ability to modulate tissue maintenance; (10) ability to modulate neural development, e.g., axonal growth, synaptogenesis, neurite  
25 outgrowth, membrane excitability and/or guidance; or (11) ability to bind a divalent cation, e.g.,  $Zn^{2+}$ ,  $Mg^{2+}$ ,  $Cd^{2+}$ ,  $Mn^{2+}$ , and/or preferably a  $Ca^{2+}$  ion.

Based on the above-described sequence similarities, the 33410 molecules of the present invention are predicted to have similar biological activities as carboxylesterase family members, in particular neuroligin proteins. Thus, the 33410 molecules can act as  
30 novel diagnostic targets and therapeutic agents for controlling cell proliferative and cell differentiative disorders, as well as neural disorder (e.g., neurodegenerative disorders including CNS disorders).

The 33410 protein may be involved in disorders characterized by aberrant activity of the cells in which it is expressed. 33410 is expressed in cells and tissues derived from heart, arteries, kidney, brain (e.g., cortex and hypothalamus), spinal cord and ovaries (Figures 6-7). Accordingly, the 33410 molecules can serve as novel diagnostic targets and therapeutic agents for controlling disorders involving the cells or tissues where they are expressed. For example, the 33410 molecules can serve as novel diagnostic targets and therapeutic agents for controlling disorders of cell proliferation, cell differentiation, angiogenesis, organogenesis, and cell signaling.

Disorders involving the brain include, but are not limited to, disorders involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2, *Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination;

degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including

5 striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as

10 leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B<sub>1</sub>) deficiency and vitamin B<sub>12</sub> deficiency, neurologic sequelae of

15 metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma,

20 oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor

25 (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

The polypeptides and nucleic acids of the invention can also be used to treat, prevent, and/or diagnose cancers and neoplastic conditions. Examples of cellular

30 proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g., leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or  
 5 may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic  
 10 hyperproliferative cells include proliferation of cells associated with wound repair.

The terms "cancer" or "neoplasms" include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell  
 15 carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas  
 20 include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

25 The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

Examples of cancers or neoplastic conditions, include, but are not limited to, a fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma,  
 30 synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastric cancer, esophageal cancer, rectal cancer, pancreatic cancer, ovarian cancer, prostate cancer, uterine cancer, cancer of the head and neck, skin cancer, brain cancer, squamous cell carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma,

cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, 5 craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, or Kaposi sarcoma.

Examples of cellular proliferative and/or differentiative disorders of the breast include, but are not limited to, proliferative breast disease including, e.g., epithelial 10 hyperplasia, sclerosing adenosis, and small duct papillomas; tumors, e.g., stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal 15 carcinoma, invasive lobular carcinoma, medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms. Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

Examples of cellular proliferative and/or differentiative disorders of the lung include, 20 but are not limited to, bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant 25 mesothelioma.

Examples of cellular proliferative and/or differentiative disorders of the colon include, but are not limited to, non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

Examples of cellular proliferative and/or differentiative disorders of the liver include, 30 but are not limited to, nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

Examples of cellular proliferative and/or differentiative disorders of the ovary include, but are not limited to, ovarian tumors such as, tumors of coelomic epithelium,

serous tumors, mucinous tumors, endometrial tumors, clear cell adenocarcinoma, cystadenofibroma, Brenner tumor, surface epithelial tumors; germ cell tumors such as mature (benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stromal tumors such as, 5 granulosa-theca cell tumors, thecoma-fibromas, androblastomas, hilus cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

The 33410 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of hematopoietic neoplastic disorders. Additional examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term 10 "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias, e.g., erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia 15 (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HCL) and Waldenstrom's 20 macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Sternberg disease.

As the 33410 mRNA is expressed in the normal heart, artery, spinal cord, kidney, 25 brain, and ovary, it is likely that 33410 molecules of the present invention are involved in disorders characterized by aberrant activity of these cells. Thus, the 33410 molecules can act as novel diagnostic targets and therapeutic agents for controlling disorders involving aberrant activity of these cells. For example, modulators of 33410 polypeptide or nucleic acid activity or expression can be used to treat or prevent endothelial cell disorders, and 30 more broadly cardiovascular or blood vessel disorders.

The 33410 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 thereof are collectively referred to as "polypeptides or proteins of the invention" or "33410 polypeptides or proteins". Nucleic acid molecules encoding such

polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "33410 nucleic acids." 33410 molecules refer to 33410 nucleic acids, polypeptides, and antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules that are separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules that are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences that naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and non-aqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C;



and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

5 Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or 3, corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural  
10 protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 33410 protein, preferably a mammalian 33410 protein, and can further include non-coding regulatory sequences, and introns.

15 An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of 33410 protein having less than about 30%, 20%, 10% and more preferably  
20 5% (by dry weight), of non-33410 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-33410 chemicals. When the 33410 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume  
25 of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 33410 (e.g., the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_)  
30 without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, e.g., those

present in the carboxylesterase domain, are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 33410 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 33410 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 33410 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a 33410 protein includes a fragment of a 33410 protein that participates in an interaction between a 33410 molecule and a non-33410 molecule. Biologically active portions of a 33410 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 33410 protein, e.g., the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length 33410 proteins, and exhibit at least one activity of a 33410 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 33410 protein, e.g., (1) binding to cell surface ligand, e.g., neurexins; (2) acting as a cell surface receptor; (3) possessing cell adhesion properties; (4) mediating cell-cell interactions between, e.g., neurons; (5) regulating inter-neuronal recognition pathways for axon pathfinding; (6) regulating neuritogenesis; or (7) binding of a divalent cation, e.g.,  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cd}^{2+}$  and/or  $\text{Mn}^{2+}$ . A biologically active portion of a 33410 protein can be a polypeptide that is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 33410 protein can be used as targets for

developing agents that modulate a 33410 mediated activity, e.g., an activity as described herein.

Particular 33410 polypeptides of the present invention have an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:2. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 are termed substantially identical.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1 or 3 are termed substantially identical.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 33410 amino acid sequence of SEQ ID NO:2 having 560 amino acid residues, at least [30%] 168, preferably at least [40%] 224, more preferably at least [50%] 280, even more preferably at

least [60%] 336, and even more preferably at least [70%] 392, [80%] 448, or [90%] 504 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10.

5 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject," as used herein, refers to human and non-human animals. The term "non-human animals" of the invention includes all vertebrates, e.g., mammals, such as non-human primates (particularly higher primates), sheep, dog, rodent (e.g., mouse or rat), guinea pig, goat, pig, cat, rabbits, cow, and non-mammals, such as chickens, amphibians, reptiles, etc. In a preferred embodiment, the subject is a human. In another embodiment, the subject is an experimental animal or animal suitable as a disease model.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule that encodes a 33410 polypeptide described herein, e.g., a full-length 33410 protein or a fragment thereof, e.g., a biologically active portion of 33410 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify nucleic acid molecule encoding a polypeptide of the invention, 33410 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human 33410 protein (i.e., "the coding region" of SEQ ID NO:1, as shown in SEQ ID NO:3), as well as 5' untranslated sequences. Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to a fragment of the protein from about amino acid 42 to 601 of SEQ ID NO:2.

In another embodiment, an isolated nucleic acid molecule of the invention includes a  
20 nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID  
NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid  
deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide  
sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently  
complementary to the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the  
25 nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession  
Number \_\_\_\_\_ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1  
or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as  
Accession Number \_\_\_\_\_, thereby forming a stable duplex.

- 29 -

5

5

5

15

25

30

30

nucleotides of a sense or antisense sequence of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid that encodes a carboxylesterase domain from about amino acid 42 to 601 of SEQ ID NO:2, a carboxylesterase type-B signature 2 domain from about amino acid 139 to 149 of SEQ ID NO:2, or a transmembrane domain from about amino acid 676 to 698 of SEQ ID NO:2.

In another embodiment a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a 33410 sequence, e.g., a domain, region, site or other sequence described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a naturally occurring variant. For example, primers suitable for amplifying all or a portion of any of the following regions are provided: a carboxylesterase domain from about amino acid 42 to 601 of SEQ ID NO:2; a carboxylesterase type-B signature 2 domain from about amino acid 139 to 149 of SEQ ID NO:2; or a transmembrane domain from about amino acid 676 to 698 of SEQ ID NO:2.

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of a 33410 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, which encodes a polypeptide having a 33410 biological activity (e.g., the biological activities of the 33410 proteins are described herein), expressing the encoded portion of the 33410 protein (e.g., by recombinant expression *in vitro*) and



5

10

15

20

30

In a preferred embodiment, a nucleic acid fragment differs by at least 1, 2, 3, 10, 20, or more nucleotides from the sequence of Genbank accession number V59639. Differ can include differing in length or sequence identity. E.g., a nucleic acid fragment can: include one or more nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of  
 5 nucleotides 3758-4297; not include all of the nucleotides of V59639, e.g., can be one or more nucleotides shorter (at one or both ends) than the sequence of V59639; or can differ by one or more nucleotides in the region of overlap.

In a preferred embodiment, a nucleic acid fragment differs by at least 1, 2, 3, 10, 20, or more nucleotides from the sequence of Genbank accession number AAA97870. Differ  
 10 can include differing in length or sequence identity. E.g., a nucleic acid fragment can: include one or more nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of nucleotides 420-2372; not include all of the nucleotides of AAA97870, e.g., can be one or more nucleotides shorter (at one or both ends) than the sequence of AAA97870; or can differ by one or more nucleotides in the region of overlap.

In a preferred embodiment, a nucleic acid fragment differs by at least 1, 2, 3, 10, 20, or more nucleotides from the sequence of Genbank accession number BAA92604. Differ  
 15 can include differing in length or sequence identity. E.g., a nucleic acid fragment can: include one or more nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of nucleotides 1275-2924; not include all of the nucleotides of BAA9260, e.g., can be one or  
 20 more nucleotides shorter (at one or both ends) than the sequence of BAA9260; or can differ by one or more nucleotides in the region of overlap.

In a preferred embodiment, a nucleic acid fragment differs by at least 1, 2, 3, 10, 20, or more nucleotides from a sequence in WO 01/27277.7. Differ can include differing in length or sequence identity. E.g., a nucleic acid fragment can: include one or more  
 25 nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of nucleotides 420-2320; not include all of the nucleotides of a sequence in WO 01/27277.7, e.g., can be one or more nucleotides shorter (at one or both ends) than a sequence in WO 01/27277.7; or can differ by one or more nucleotides in the region of overlap.

In a preferred embodiment, a nucleic acid fragment differs by at least 1, 2, 3, 10, 20,  
 30 or more nucleotides from a sequence in WO 01/27277.8. Differ can include differing in length or sequence identity. E.g., a nucleic acid fragment can: include one or more nucleotides from SEQ ID NO: 1 or SEQ ID NO:3 outside the region of nucleotides 420-2372; not include all of the nucleotides of a sequence in WO 01/27277.8, e.g., can be one or

more nucleotides shorter (at one or both ends) than a sequence in WO 01/27277.8; or can differ by one or more nucleotides in the region of overlap.

In a preferred embodiment, a nucleic acid fragment includes a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500,  
 5 1600, 1700, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_.

#### 10 33410 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid that  
 15 encodes the same 33410 proteins as those encoded by the nucleotide sequence disclosed herein). In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum  
 20 homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the inventor can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, and preferably at least 10%, or 20% of the codons have been  
 25 altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including  
 30 those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1 or 3, or the sequence in ATCC Accession Number \_\_\_, e.g., as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 2%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the nucleotide sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO 2 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 33410 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 33410 gene.

Preferred variants include those that are correlated with modulating (stimulating and/or enhancing or inhibiting) cell adhesion, cellular proliferation, differentiation, or tumorigenesis; modulating neural cell activity; binding to neurexins on neurons; acting as a cell surface receptor; mediating cell-cell interactions between neurons; regulating inter-neuronal recognition pathways for axon pathfinding; regulating neuritogenesis; or binding divalent cations, e.g.,  $Zn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cd^{2+}$  and/or  $Mn^{2+}$ .

Allelic variants of 33410, e.g., human 33410, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 33410 protein within a population that maintain the ability to bind an extracellular component or cell surface protein, e.g., neurexin. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 33410, e.g., human 33410, protein within a population that do not have the ability to bind to neurexins; act as a cell surface receptor; possess cell adhesion properties; mediate cell-cell interactions between neurons; regulate inter-neuronal recognition pathways for axon pathfinding; regulate neuritogenesis; or bind  $Zn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cd^{2+}$  and/or  $Mn^{2+}$ . Non-functional allelic variants will typically contain a non-conservative substitution, a

Moreover, nucleic acid molecules encoding other 33410 family members and, thus, which have a nucleotide sequence which differs from the 33410 sequences of SEQ ID NO:1 or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ are intended to be within the scope of the invention.

## 10 Molecules

sequence. The antisense nucleic acid can be complementary to an entire 33410 coding strand, or to only a portion thereof (e.g., the coding region of human 33410 corresponding to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 33410 (e.g., the 5' and 3' untranslated regions).

20 An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 33410 mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of 33410 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 33410 mRNA, e.g., between the -10 and +10 regions of the target gene  
25 nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

30 using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be

produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

5           The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 33410 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and  
10 then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular  
15 concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units,  
20 the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme.  
25 A ribozyme having specificity for a 33410-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 33410 cDNA disclosed herein (i.e., SEQ ID NO:1 or SEQ ID NO:3), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA  
30 can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 33410-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, 33410 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a

pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

33410 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 33410 (e.g., the 33410 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 33410 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. et al. (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15. Potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

A 33410 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. et al. (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. et al. (1996) *supra*; Perry-O'Keefe et al. *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of 33410 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 33410 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1

nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. et al. (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol et al. (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 33410 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such 15 that the molecular beacon is useful for quantitating the presence of the 33410 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi et al., U.S. Patent No. 5,854,033; Nazarenko et al., U.S. Patent No. 5,866,336, and Livak et al., U.S. Patent 5,876,930.

## Isolated 33410 Polypeptides

In another aspect, the invention features an isolated 33410 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-33410 antibodies. 33410 protein can be isolated from cells or tissue sources using standard protein purification techniques. 33410 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those that arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and post-translational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same post-translational modifications present when expressed the polypeptide is expressed in a native cell, or in systems that result in the alteration or omission of post-translational modifications present when expressed in a native cell, e.g., glycosylation or cleavage.



In a preferred embodiment, A 33410 polypeptide has one or more of the following characteristics:

- (i) it acts on carboxylic esters, e.g., acetylcholinesterase;
- (ii) it modulates cell-cell (e.g., neuron-neuron, or neuron-glia) recognition event or  
5   adhesion;
- (iii) it binds an extracellular component or cell surface protein, e.g., neurexin;
- (iv) it modulates neural developmental events, for example, membrane excitability, neurite outgrowth, and/or synaptogenesis;
- (v) it has an amino acid composition of SEQ ID NO:2;
- 10   (vi) it has an overall sequence similarity of at least 60%, preferably at least 70, more preferably at least 80, 90, or 95%, with a polypeptide of SEQ ID NO:2;
- (vii) it can be found in human tissue;
- (viii) it can be found in neural tissues, e.g., neurons;
- (ix) it has a carboxylesterase domain which is preferably about 70%, 80%, 90% or  
15   95% with amino acid residues from about 42 to 601 of SEQ ID NO:2;
- (x)    it has a carboxylesterase type-B signature 2 domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues from about 139 to 149 of SEQ ID NO:2;
- (xi)   it has a transmembrane domain which is preferably about 70%, 80%, 90% or 95% with amino acid residues from about 676 to 698 of SEQ ID NO:2; or
- 20   (xii) it has at least 1, preferably 5, and most preferably 6 of the cysteines found in the amino acid sequence of the native protein (i.e., SEQ ID NO:2).

In a preferred embodiment, the 33410 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID:2. In one embodiment, it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding  
25   sequence in SEQ ID NO:2 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are preferably differences or changes at a non-essential residue or a conservative  
30   substitution. In a preferred embodiment, the differences are not in the carboxylesterase domain (e.g., amino acids 42 to 601 of SEQ ID NO:2). In another preferred embodiment, one or more differences are in the carboxylesterase domain (e.g., amino acids 42 to 601 of SEQ ID NO:2).

5 In one embodiment, the protein includes an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or more homologous to SEQ ID NO:2.

In one embodiment, a biologically active portion of a 33410 protein includes an carboxylesterase domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 33410 protein.

In a preferred embodiment, a fragment differs by at least 1, 2, 3, 10, 20, or more amino acid residues from a sequence in WO 01/27277.7. Differ can include differing in length or sequence identity. E.g., a fragment can: include one or more amino acid residues from SEQ ID NO: 2 outside the region encoded by nucleotides 420-2320; not include all of the amino acid residues of a sequence in WO 01/27277.7, e.g., can be one or more amino acid residues shorter (at one or both ends) than a sequence in WO 01/27277.7; or can differ by one or more amino acid residues in the region of overlap.

In a preferred embodiment, a fragment differs by at least 1, 2, 3, 10, 20, or more amino acid residues from a sequence in WO 01/27277.8. Differ can include differing in length or sequence identity. E.g., a fragment can: include one or more amino acid residues from SEQ ID NO: 2 outside the region encoded by nucleotides 420-2372; not include all of the amino acid residues of a sequence in WO 01/27277.8, e.g., can be one or more amino acid residues shorter (at one or both ends) than a sequence in WO 01/27277.8; or can differ by one or more amino acid residues in the region of overlap.

### 33410 Chimeric or Fusion Proteins

In another aspect, the invention provides 33410 chimeric or fusion proteins. As used herein, a 33410 "chimeric protein" or "fusion protein" includes a 33410 polypeptide linked to a non-33410 polypeptide. A "non-33410 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 33410 protein, e.g., a protein which is different from the 33410 protein and which is derived from the same or a different organism. The 33410 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a 33410 amino acid sequence. In a preferred embodiment, a 33410 fusion protein includes at least one (or two) biologically active portion of a 33410 protein. The non-33410 polypeptide can be fused to the N-terminus or C-terminus of the 33410 polypeptide.

The fusion protein can include a moiety that has a high affinity for a ligand. For example, the fusion protein can be a GST-33410 fusion protein in which the 33410 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 33410. Alternatively, the fusion protein can be a 33410 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of 33410 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

The 33410 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 33410 fusion proteins can be used to affect the bioavailability of a 33410 substrate. 33410 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant

modification or mutation of a gene encoding a 33410 protein; (ii) mis-regulation of the 33410 gene; and (iii) aberrant post-translational modification of a 33410 protein.

Moreover, the 33410-fusion proteins of the invention can be used as immunogens to produce anti-33410 antibodies in a subject, to purify 33410 ligands and in screening assays to identify molecules that inhibit the interaction of 33410 with a 33410 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A 33410-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 33410 protein.

#### 10 Variants of 33410 Proteins

In another aspect, the invention also features a variant of a 33410 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the 33410 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a 33410 protein. An agonist of the 33410 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 33410 protein. An antagonist of a 33410 protein can inhibit one or more of the activities of the naturally occurring form of the 33410 protein by, for example, competitively modulating a 33410-mediated activity of a 33410 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 33410 protein.

Variants of a 33410 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a 33410 protein for agonist or antagonist activity.

Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of a 33410 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 33410 protein.

Variants in which a cysteine residue is added or deleted or in which a residue that is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination

with the screening assays to identify 33410 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated 33410 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to 33410 in a substrate-dependent manner. The transfected cells are then contacted with 33410 and the effect of the expression of the mutant on signaling by the 33410 substrate can be detected, e.g., by measuring the amount of binding to neurexins on neurons; the ability to act as a cell surface receptor; the cell adhesion properties; the ability to mediate cell-cell interactions between neurons; the ability to regulate inter-neuronal recognition pathways for axon pathfinding; the ability to regulate neuritogenesis; or the ability to bind  $Zn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cd^{2+}$  and/or  $Mn^{2+}$ . Plasmid DNA can then be recovered from those cells that score for inhibition, or alternatively, potentiation of signaling by the 33410 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a 33410 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring 33410 polypeptide, e.g., a naturally occurring 33410 polypeptide. The method includes: altering the sequence of a 33410 polypeptide, e.g., altering the sequence, e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a 33410 polypeptide a biological activity of a naturally occurring 33410 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a 33410 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

#### Anti-33410 Antibodies

In another aspect, the invention provides an anti-33410 antibody, or a fragment thereof (e.g., an antigen-binding fragment thereof). The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. As used herein, the term "antibody" refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein

as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDR's has been precisely defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, which are incorporated herein by reference). Each VH and VL is composed of three CDR's and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The anti-33410 antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are interconnected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 kD or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to the antigen, e.g., 33410 polypeptide or fragment

thereof. Examples of antigen-binding fragments of the anti-33410 antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The anti-33410 antibody can be a polyclonal or a monoclonal antibody. In other embodiments, the antibody can be recombinantly produced, *e.g.*, produced by phage display or by combinatorial methods.

Phage display and combinatorial methods for generating anti-33410 antibodies are known in the art (as described in, *e.g.*, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* International Publication No. WO 92/18619; Dower *et al.* International Publication No. WO 91/17271; Winter *et al.* International Publication WO 92/20791; Markland *et al.* International Publication No. WO 92/15679; Breitling *et al.* International Publication WO 93/01288; McCafferty *et al.* International Publication No. WO 92/01047; Garrard *et al.* International Publication No. WO 92/09690; Ladner *et al.* International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982, the contents of all of which are incorporated by reference herein).

In one embodiment, the anti-33410 antibody is a fully human antibody (e.g., an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, e.g., a rodent (mouse or rat), goat, primate (e.g., monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Method of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuaillon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

An anti-33410 antibody can be one in which the variable region, or a portion thereof, e.g., the CDR's, are generated in a non-human organism, e.g., a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibodies generated in a non-human organism, e.g., a rat or mouse, and then modified, e.g., in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).



A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDR's (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDR's may be replaced with non-human CDR's. It is only necessary to replace the number of CDR's required for binding of the humanized antibody to a 33410 or a fragment thereof. Preferably, the donor will be a rodent antibody, e.g., a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDR's is called the "donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the donor immunoglobulin is a non-human (e.g., rodent). The acceptor framework is a naturally occurring (e.g., a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

An antibody can be humanized by methods known in the art. Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a 33410 polypeptide or fragment thereof. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Humanized or CDR-grafted antibodies can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDR's of an immunoglobulin chain can be replaced. See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference. Å

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. Preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a humanized antibody will have framework residues identical to the donor framework residue or to another amino acid other than the recipient framework residue. To generate such antibodies, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., US 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of US 5,585,089, the e.g., columns 12-16 of US 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

In preferred embodiments an antibody can be made by immunizing with purified 33410 antigen, or a fragment thereof, e.g., a fragment described herein, membrane associated antigen, tissue, e.g., crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions, e.g., membrane fractions.

A full-length 33410 protein or, antigenic peptide fragment of 33410 can be used as an immunogen or can be used to identify anti-33410 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 33410 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of 33410. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues,

even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 33410 which include, for example, residues 330-350, 480-505, or 695-720 of SEQ ID NO:2 can be used to make, e.g., antibodies against hydrophilic regions of the 33410 protein or used as immunogens or to characterize the specificity of an antibody. Similarly, a fragment of 33410 which include, for example, residues 60-72, 260-277, or 780-793 of SEQ ID NO:2 can be used to make an antibody against a hydrophobic region of the 33410 protein; a fragment of 33410 which include residues 42-601 of SEQ ID NO:2 can be used to make an antibody against an extracellular region of the 33410 protein; a fragment of 33410 which include residues 699-835 of SEQ ID NO:2 can be used to make an antibody against an intracellular region of the 33410 protein; a fragment of 33410 which include residues 42-601 or 139-149 of SEQ ID NO:2 can be used to make an antibody against a carboxylesterase region of the 33410 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Antibodies which bind only native 33410 protein, only denatured or otherwise non-native 33410 protein, or which bind both, are within the invention. Antibodies with linear or conformational epitopes are within the invention. Conformational epitopes can sometimes be identified by identifying antibodies that bind to native but not denatured 33410 protein.

Preferred epitopes encompassed by the antigenic peptide are regions of 33410 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 33410 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 33410 protein and are thus likely to constitute surface residues useful for targeting antibody production.

The anti-33410 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. *et al.* (1999) *Ann N Y Acad Sci* 880:263-80; and Reiter, Y. (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 33410 protein.

In a preferred embodiment the antibody has: effector function; and can fix complement. In other embodiments the antibody does not; recruit effector cells; or fix complement.

In a preferred embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example., it is a isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

The antibody can be coupled to a toxin, e.g., a polypeptide toxin, e.g, ricin or diphtheria toxin or active fragment hereof, or a radionuclide, or imaging agent, e.g. a radioactive, enzymatic, or other, e.g., imaging agent, e.g., a NMR contrast agent. Labels which produce detectable radioactive emissions or fluorescence are preferred.

An anti-33410 antibody (e.g., monoclonal antibody) can be used to isolate 33410 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-33410 antibody can be used to detect 33410 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-33410 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

The invention also includes a nucleic acid that encodes an anti-33410 antibody, e.g., an anti-33410 antibody described herein. Also included are vectors which include the nucleic acid and cells transformed with the nucleic acid, particularly cells which are useful for producing an antibody, e.g., mammalian cells, e.g. CHO or lymphatic cells.

The invention also includes cell lines, e.g., hybridomas, which make an anti-33410 antibody, e.g., and antibody described herein, and method of using said cells to make a 33410 antibody.

## 5 Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can  
10 be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include a 33410 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed.  
15 The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the  
20 like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., 33410 proteins, mutant forms of 33410 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression  
25 of 33410 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and  
30 translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded

therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 33410 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 33410 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells that are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six weeks).

One strategy used to maximize recombinant protein expression in *E. coli* is to express the protein in a host strain with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 33410 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 33410 nucleic acid molecule within a recombinant expression vector or a 33410 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell, but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 33410 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

5 Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

10 A host cell of the invention can be used to produce (i.e., express) a 33410 protein. Accordingly, the invention further provides methods for producing a 33410 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 33410 protein has been introduced) in a suitable medium such that a 33410 protein is produced. In another  
15 embodiment, the method further includes isolating a 33410 protein from the medium or the host cell.

In another aspect, the invention features a cell or purified preparation of cells which include a 33410 transgene, or which otherwise misexpress 33410. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells,  
20 or pig cells. In preferred embodiments, the cell or cells include a 33410 transgene, e.g., a heterologous form of a 33410, e.g., a gene derived from humans (in the case of a non-human cell). The 33410 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene that misexpress an endogenous 33410, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can  
25 serve as a model for studying disorders that are related to mutated or mis-expressed 33410 alleles or for use in drug screening.

In another aspect, the invention features a human cell, e.g., a neurological cell, transformed with nucleic acid that encodes a subject 33410 polypeptide.

Also provided are cells, preferably human cells, e.g., human neurological cells (e.g.,  
30 neurons), in which an endogenous 33410 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 33410 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell



such that the inserted regulatory element is operably linked to the endogenous 33410 gene. For example, an endogenous 33410 gene that is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published in May 16, 1991.

### Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 33410 protein and for identifying and/or evaluating modulators of 33410 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 33410 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 33410 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 33410 transgene in its genome and/or expression of 33410 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 33410 protein can further be bred to other transgenic animals carrying other transgenes.

33410 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs  
 5 produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed, e.g., below.

#### Uses

10 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to  
 15 express a 33410 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a 33410 mRNA (e.g., in a biological sample) or a genetic alteration in a 33410 gene, and to modulate 33410 activity, as described further below. The 33410 proteins can be used to treat disorders characterized by insufficient or excessive production of a 33410 substrate or production of 33410 inhibitors. In addition, the 33410  
 20 proteins can be used to screen for naturally occurring 33410 substrates, to screen for drugs or compounds which modulate 33410 activity, as well as to treat disorders characterized by insufficient or excessive production of 33410 protein or production of 33410 protein forms which have decreased, aberrant or unwanted activity compared to 33410 wild type protein (e.g., neurological disorders and/or carcinomas). Moreover, the anti-33410 antibodies of the  
 25 invention can be used to detect and isolate 33410 proteins, regulate the bioavailability of 33410 proteins, and modulate 33410 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject 33410 polypeptide is provided. The method includes: contacting the compound with the subject 33410 polypeptide; and evaluating ability of the compound to interact with,  
 30 e.g., to bind or form a complex with the subject 33410 polypeptide. This method can be performed in vitro, e.g., in a cell free system, or in vivo, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules that interact with

subject 33410 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 33410 polypeptide. Screening methods are discussed in more detail below.

### Screening Assays

5           The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to 33410 proteins, have a stimulatory or inhibitory effect on, for example, 33410 expression or 33410 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a  
10   33410 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., 33410 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

          In one embodiment, the invention provides assays for screening candidate or test  
15   compounds that are substrates of a 33410 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate the activity of a 33410 protein or polypeptide or a biologically active portion thereof.

          The test compounds of the present invention can be obtained using any of the  
20   numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann, R.N. et al. *J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase  
25   libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

30           Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed.*

*Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate activity is determined. Determining the ability of the test compound to modulate activity can be accomplished by monitoring, for example, binding to neurexins on neurons; the ability to act as a cell surface receptor; cell adhesion properties; the ability to mediate cell-cell interactions between neurons; the ability to regulate inter-neuronal recognition pathways for axon pathfinding; the ability to regulate neuritogenesis; or binding  $Zn^{2+}$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Cd^{2+}$  and/or  $Mn^{2+}$ . The cell, for example, can be of mammalian origin, e.g., human.

The ability of the test compound to modulate binding to a compound, e.g., a substrate, or to bind to can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate binding to a substrate in a complex. For example, compounds (e.g., substrates) can be labeled with  $^{125}I$ ,  $^{35}S$ ,  $^{14}C$ , or  $^3H$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (e.g., a substrate) to interact with with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with without

the labeling of either the compound or the 33410. McConnell, H. M. et al. (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 33410.

In yet another embodiment, a cell-free assay is provided in which a 33410 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 33410 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 33410 proteins to be used in assays of the present invention include fragments that participate in interactions with non-33410 molecules, e.g., fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (e.g., 33410 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be

assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

5 In another embodiment, determining the ability of the 33410 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo et al. (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants  
10 (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a  
15 solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 33410, an anti-33410 antibody or its target  
20 molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 33410 protein, or interaction of a 33410 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes,  
25 and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/33410 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test  
30 compound or the test compound and either the non-adsorbed target protein or 33410 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the

case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 33410 binding or activity determined using standard techniques.

Other techniques for immobilizing either a 33410 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 33410 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 33410 protein or target molecules but which do not interfere with binding of the 33410 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 33410 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 33410 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 33410 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., *Trends Biochem Sci* 1993 Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. et al., eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. et al.,

5

10

15

30





complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 33410 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Biotechniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 33410 ("33410-binding proteins" or "33410-bp") and are involved in 33410 activity. Such 33410-bps can be activators or inhibitors of signals by the 33410 proteins or 33410 targets as, for example, downstream elements of a 33410-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 33410 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g.,

5

15

25

30

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 33410 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

10           The 33410 nucleotide sequences or portions thereof can be used to map the location of the 33410 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 33410 sequences with genes associated with disease.

A panel of somatic cell hybrids in which each cell line contains either a single human  
20 chromosome or a small number of human chromosomes, and a full set of mouse  
chromosomes, can allow easy mapping of individual genes to specific human chromosomes.  
(D'Eustachio P. et al. (1983) *Science* 220:919-924).

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable

amount of time. For a review of this technique, see Verma et al., Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to non-coding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available online through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. et al. (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 33410 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### Tissue Typing

33410 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 33410 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 33410 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

#### Use of Partial 33410 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for

identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

5           The 33410 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 33410 probes can be used to identify tissue by species and/or by organ type.

10           In a similar fashion, these reagents, e.g., 33410 primers or probes can be used to screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

#### Predictive Medicine

15           The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

            Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes 33410.

20           Such disorders include, e.g., a disorder associated with the misexpression of 33410 gene; a neoplasia or a disorder of the neurological system.

            The method includes one or more of the following:

            detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 33410 gene, or detecting the presence or absence of a mutation  
25   in a region which controls the expression of the gene, e.g., a mutation in the 5' control region;

            detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 33410 gene;

            detecting, in a tissue of the subject, the misexpression of the 33410 gene, at the  
30   mRNA level, e.g., detecting a non-wild type level of a mRNA;

            detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of a 33410 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 33410 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1, or naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the 33410 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 33410 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 33410.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of a 33410 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 33410 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

## Diagnostic and Prognostic Assays

Diagnostic and prognostic assays of the invention include method for assessing the expression level of 33410 molecules and for identifying variations and mutations in the sequence of 33410 molecules.

## Expression Monitoring and Profiling.

The presence, level, or absence of 33410 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 33410 protein or



nucleic acid (e.g., mRNA, genomic DNA) that encodes 33410 protein such that the presence of 33410 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum.

- 5 The level of expression of the 33410 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 33410 genes; measuring the amount of protein encoded by the 33410 genes; or measuring the activity of the protein encoded by the 33410 genes.

The level of mRNA corresponding to the 33410 gene in a cell can be determined  
10 both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can  
15 hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 33410 nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 33410 mRNA or genomic DNA. The probe can be disposed on an address of an array, e.g.,  
20 an array described below. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the  
25 probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array described below. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 33410 genes.

The level of mRNA in a sample that is encoded by one of 33410 can be evaluated  
30 with nucleic acid amplification, e.g., by RT-PCR (Mullis (1987) U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, (1989), *Proc. Natl. Acad. Sci. USA*

86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of

5 nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide

10 sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 33410 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a

15 compound or agent capable of detecting 33410 mRNA, or genomic DNA, and comparing the presence of 33410 mRNA or genomic DNA in the control sample with the presence of 33410 mRNA or genomic DNA in the test sample. In still another embodiment, serial analysis of gene expression, as described in U.S. Patent No. 5,695,937, is used to detect 33410 transcript levels.

20 A variety of methods can be used to determine the level of protein encoded by 33410. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g.,

25 Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

30 The detection methods can be used to detect 33410 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 33410 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo*

techniques for detection of 33410 protein include introducing into a subject a labeled anti-33410 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In another embodiment, the sample is labeled, e.g., biotinylated and then contacted to the antibody, e.g., an anti-33410 antibody positioned on an antibody array (as described below). The sample can be detected, e.g., with avidin coupled to a fluorescent label.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 33410 protein, and comparing the presence of 33410 protein in the control sample with the presence of 33410 protein in the test sample.

The invention also includes kits for detecting the presence of 33410 in a biological sample. For example, the kit can include a compound or agent capable of detecting 33410 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 33410 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein-stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 33410 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 33410 expression or activity is identified. A test sample is obtained from a subject and 33410 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 33410 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 33410 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 33410 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disease or disorder associated with misexpressed or aberrant or unwanted 33410 expression or activity.

In another aspect, the invention features a computer medium having a plurality of digitally encoded data records. Each data record includes a value representing the level of expression of 33410 in a sample, and a descriptor of the sample. The descriptor of the sample can be an identifier of the sample, a subject from which the sample was derived (e.g., a patient), a diagnosis, or a treatment (e.g., a preferred treatment). In a preferred embodiment, the data record further includes values representing the level of expression of genes other than 33410 (e.g., other genes associated with a 33410-disorder, or other genes on an array). The data record can be structured as a table, e.g., a table that is part of a database such as a relational database (e.g., a SQL database of the Oracle or Sybase database environments).

Also featured is a method of evaluating a sample. The method includes providing a sample, e.g., from the subject, and determining a gene expression profile of the sample, wherein the profile includes a value representing the level of 33410 expression. The method can further include comparing the value or the profile (i.e., multiple values) to a reference value or reference profile. The gene expression profile of the sample can be obtained by any of the methods described herein (e.g., by providing a nucleic acid from the sample and contacting the nucleic acid to an array). The method can be used to diagnose a disease or disorder associated with misexpressed or aberrant or unwanted 33410 expression

or activity in a subject wherein an increase or a decrease in 33410 expression is an indication that the subject has or is disposed to having a disease or disorder associated with misexpressed or aberrant or unwanted 33410 expression or activity. The method can be used to monitor a treatment for a disease or disorder associated with misexpressed or aberrant or unwanted 33410 expression or activity in a subject. For example, the gene expression profile can be determined for a sample from a subject undergoing treatment. The profile can be compared to a reference profile or to a profile obtained from the subject prior to treatment or prior to onset of the disorder (see, e.g., Golub *et al.* (1999) *Science* 286:531).

In yet another aspect, the invention features a method of evaluating a test compound (see also, "Screening Assays", above). The method includes providing a cell and a test compound; contacting the test compound to the cell; obtaining a subject expression profile for the contacted cell; and comparing the subject expression profile to one or more reference profiles. The profiles include a value representing the level of 33410 expression. In a preferred embodiment, the subject expression profile is compared to a target profile, e.g., a profile for a normal cell or for desired condition of a cell. The test compound is evaluated favorably if the subject expression profile is more similar to the target profile than an expression profile obtained from a cell not contacted with the test compound.

In another aspect, the invention features a method of evaluating a subject. The method includes: a) obtaining a sample from a subject, e.g., from a caregiver, e.g., a caregiver who obtains the sample from the subject; b) determining a subject expression profile for the sample. Optionally, the method further includes either or both of steps: c) comparing the subject expression profile to one or more reference expression profiles; and d) selecting the reference profile most similar to the subject reference profile. The subject expression profile and the reference profiles include a value representing the level of 33410 expression. A variety of routine statistical measures can be used to compare two reference profiles. One possible metric is the length of the distance vector that is the difference between the two profiles. Each of the subject and reference profile is represented as a multi-dimensional vector, wherein each dimension is a value in the profile.

The method can further include transmitting a result to a caregiver. The result can be the subject expression profile, a result of a comparison of the subject expression profile with another profile, a most similar reference profile, or a descriptor of any of the aforementioned. The result can be transmitted across a computer network, e.g., the result

can be in the form of a computer transmission, e.g., a computer data signal embedded in a carrier wave.

Also featured is a computer medium having executable code for effecting the following steps: receive a subject expression profile; access a database of reference expression profiles; and either i) select a matching reference profile most similar to the subject expression profile or ii) determine at least one comparison score for the similarity of the subject expression profile to at least one reference profile. The subject expression profile, and the reference expression profiles each include a value representing the level of 33410 expression.

#### Arrays and Uses Thereof

In another aspect, the invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a 33410 molecule (e.g., a 33410 nucleic acid or a 33410 polypeptide). The array can have a density of at least than 10, 50, 100, 200, 500, 1,000, 2,000, or 10,000 or more addresses/cm<sup>2</sup>, and ranges between. In a preferred embodiment, the plurality of addresses includes at least 10, 100, 500, 1,000, 5,000, 10,000, 50,000 addresses. In a preferred embodiment, the plurality of addresses includes equal to or less than 10, 100, 500, 1,000, 5,000, 10,000, or 50,000 addresses. The substrate can be a two-dimensional substrate such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can be disposed on the array.

In a preferred embodiment, at least one address of the plurality includes a nucleic acid capture probe that hybridizes specifically to a 33410 nucleic acid, e.g., the sense or anti-sense strand. In one preferred embodiment, a subset of addresses of the plurality of addresses has a nucleic acid capture probe for 33410. Each address of the subset can include a capture probe that hybridizes to a different region of a 33410 nucleic acid. In another preferred embodiment, addresses of the subset include a capture probe for a 33410 nucleic acid. Each address of the subset is unique, overlapping, and complementary to a different variant of 33410 (e.g., an allelic variant, or all possible hypothetical variants). The array can be used to sequence 33410 by hybridization (see, e.g., U.S. Patent No. 5,695,940).

An array can be generated by various methods, e.g., by photolithographic methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g.,

directed-flow methods as described in U.S. Patent No. 5,384,261), pin-based methods (e.g., as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

In another preferred embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to a 33410 polypeptide or fragment thereof. The polypeptide can be a naturally occurring interaction partner of 33410 polypeptide. Preferably, the polypeptide is an antibody, e.g., an antibody described herein (see "Anti-33410 Antibodies," above), such as a monoclonal antibody or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of 33410. The method includes providing an array as described above; contacting the array with a sample and detecting binding of a 33410-molecule (e.g., nucleic acid or polypeptide) to the array. In a preferred embodiment, the array is a nucleic acid array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of 33410. If a sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with 33410. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on 33410 expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

In another embodiment, cells are contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect

on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can be probed with the array. Such analysis can identify and/or characterize the development of a 33410-associated disease or disorder; and processes, such as a cellular transformation associated with a 33410-associated disease or disorder. The method can also evaluate the treatment and/or progression of a 33410-associated disease or disorder

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including 33410) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has disposed thereon a 33410 polypeptide or fragment thereof. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt *et al.* (2000). *Nature Biotech.* 18, 989-994; Lueking *et al.* (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S.L. (2000). *Science* 289, 1760-1763; and WO 99/51773A1. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60, 70, 80, 85, 90, 95 or 99 % identical to a 33410 polypeptide or fragment thereof. For example, multiple variants of a 33410 polypeptide (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality. Addresses in addition to the address of the plurality can be disposed on the array.

The polypeptide array can be used to detect a 33410 binding compound, e.g., an antibody in a sample from a subject with specificity for a 33410 polypeptide or the presence of a 33410-binding protein or ligand.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (e.g., ascertaining the effect of 33410 expression on the expression of other genes). This provides, for example, for a



In another aspect, the invention features a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express 33410 or from a cell or subject in which a 33410 mediated response has been elicited, e.g., by contact of the cell with 33410 nucleic acid or protein, or administration to the cell or subject 33410 nucleic acid or protein; providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express 33410 (or does not express as highly as in the case of the 33410 positive plurality of capture probes) or from a cell or subject which in which a 33410 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 33410 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

of the 33410 positive plurality of capture probes) or from a cell or subject which in which a 33410 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); and comparing the binding of the first sample with the binding of the second sample. Binding, e.g., in the case of a nucleic acid, hybridization with a capture  
 5 probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody. The same array can be used for both samples or different arrays can be used. If different arrays are used the plurality of addresses with capture probes should be present on both arrays.

In another aspect, the invention features a method of analyzing 33410, e.g., analyzing  
 10 structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 33410 nucleic acid or amino acid sequence; comparing the 33410 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze 33410.

#### 15 Detection of Variations or Mutations

The methods of the invention can also be used to detect genetic alterations in a 33410 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 33410 protein activity or nucleic acid expression, such as a neurological disorder and/or carcinomas. In preferred embodiments, the methods include  
 20 detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 33410-protein, or the mis-expression of the 33410 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 33410 gene; 2) an addition of one or more nucleotides to a  
 25 33410 gene; 3) a substitution of one or more nucleotides of a 33410 gene, 4) a chromosomal rearrangement of a 33410 gene; 5) an alteration in the level of a messenger RNA transcript of a 33410 gene, 6) aberrant modification of a 33410 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 33410 gene, 8) a non-wild type level of a 33410-protein, 9)  
 30 allelic loss of a 33410 gene, and 10) inappropriate post-translational modification of a 33410-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the

latter of which can be particularly useful for detecting point mutations in the 33410-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 33410 gene under  
5 conditions such that hybridization and amplification of the 33410-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein. Alternatively,  
10 other amplification methods described herein or known in the art can be used.

In another embodiment, mutations in a 33410 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel  
15 electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 33410 can be identified by hybridizing a  
20 sample and control nucleic acids, e.g., DNA or RNA, two-dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. A probe can be complementary to a region of a 33410 nucleic acid or a putative variant (e.g., allelic variant) thereof. A probe can have one or more mismatches to a region of a  
25 33410 nucleic acid (e.g., a destabilizing mismatch). The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 33410 can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a  
30 first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of

specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 33410 gene and detect mutations by comparing the sequence of the sample 33410 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

Other methods for detecting mutations in the 33410 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242; Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295).

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 33410 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 33410 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 33410 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). A further method of detecting point mutations is the chemical ligation of oligonucleotides as described in Xu *et al.* ((2001) *Nature Biotechnol.* 19:148). Adjacent oligonucleotides, one of which selectively anneals to the query site, are ligated together if the nucleotide at the query site of the sample nucleic acid is complementary to the query oligonucleotide; ligation can be monitored, e.g., by fluorescent dyes coupled to the oligonucleotides.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

In another aspect, the invention features a set of oligonucleotides. The set includes a plurality of oligonucleotides, each of which is at least partially complementary (e.g., at least

50%, 60%, 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 99% complementary) to a 33410 nucleic acid.

In a preferred embodiment the set includes a first and a second oligonucleotide. The first and second oligonucleotide can hybridize to the same or to different locations of SEQ ID NO: 1 or the complement of SEQ ID NO:1. Different locations can be different but overlapping or non-overlapping on the same strand. The first and second oligonucleotide can hybridize to sites on the same or on different strands.

The set can be useful, e.g., for identifying SNP's, or identifying specific alleles of 33410. In a preferred embodiment, each oligonucleotide of the set has a different nucleotide at an interrogation position. In one embodiment, the set includes two oligonucleotides, each complementary to a different allele at a locus, e.g., a biallelic or polymorphic locus.

In another embodiment, the set includes four oligonucleotides, each having a different nucleotide (e.g., adenine, guanine, cytosine, or thymidine) at the interrogation position. The interrogation position can be a SNP or the site of a mutation. In another preferred embodiment, the oligonucleotides of the plurality are identical in sequence to one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotide that hybridizes to one allele provides a signal that is distinguishable from an oligonucleotide that hybridizes to a second allele. In still another embodiment, at least one of the oligonucleotides of the set has a nucleotide change at a position in addition to a query position, e.g., a destabilizing mutation to decrease the  $T_m$  of the oligonucleotide. In another embodiment, at least one oligonucleotide of the set has a non-natural nucleotide, e.g., inosine. In a preferred embodiment, the oligonucleotides are attached to a solid support, e.g., to different addresses of an array or to different beads or nanoparticles.

In a preferred embodiment the set of oligonucleotides can be used to specifically amplify, e.g., by PCR, or detect, a 33410 nucleic acid.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 33410 gene.

## Use of 33410 Molecules as Surrogate Markers

The 33410 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 33410 molecules of the invention may be detected, and may be correlated with one or more biological states in vivo. For example, the 33410 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a “surrogate marker” is an objective biochemical marker that correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 33410 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker that correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such

that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a 33410 marker) transcription or expression, the amplified marker may be in a quantity that is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-33410 antibodies may be employed in an immune-based detection system for a 33410 protein marker, or 33410-specific radiolabeled probes may be used to detect a 33410 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 33410 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker that correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35:1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 33410 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 33410 DNA may correlate with a 33410 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.



## **Pharmaceutical Compositions**

The nucleic acid and polypeptides, fragments thereof, as well as anti-33410 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the

5 nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

10 A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile

15 diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose.

20 pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous

25 preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the

30 contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a

coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including an agent in the composition that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such

compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography. As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration are often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is

described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

The present invention encompasses agents that modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine,

lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see US Patent No. 5,208,020), CC-1065 (see US Patent Nos. 5,475,092, 5,585,499, 5,846,545) and analogs or homologs thereof. and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response; the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

#### Methods of Treatment

5           The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 33410 expression or activity. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who  
10       has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

15           With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term  
20       refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 33410 molecules of the present invention or 33410 modulators according to that individual's drug response genotype. Pharmacogenomics allows a  
25       clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

          In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 33410 expression or activity, by  
30       administering to the subject a 33410 or an agent which modulates 33410 expression or at least one 33410 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 33410 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a

prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 33410 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of 33410 aberrance, for example, a 33410, 33410 agonist or 33410 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some 33410 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

The 33410 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, and neurodegenerative disorders as described above, as well as immune or inflammatory disorders, cardiovascular disorders, disorders associated with bone metabolism, liver disorders, viral diseases, pain or metabolic disorders.

The 33410 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune disorders. Examples of hematopoietic disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

Examples of disorders involving the heart or "cardiovascular disorder" include, but are not limited to, a disease, disorder, or state involving the cardiovascular system, *e.g.*, the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an



Aberrant expression and/or activity of 33410 molecules may mediate disorders associated with bone metabolism. "Bone metabolism" refers to direct or indirect effects in the formation or degeneration of bone structures, e.g., bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and phosphate. This term also includes activities mediated by 33410 molecules effects in bone cells, e.g. osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 33410 molecules may support different activities of bone resorbing osteoclasts such as the stimulation of differentiation of monocytes and mononuclear phagocytes into osteoclasts. Accordingly, 33410 molecules that modulate the production of bone cells can influence bone formation and degeneration, and thus may be used to treat bone disorders. Examples of such disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-convulsant treatment, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk fever.

25 extracellular matrix accompanied by the collapse and condensation of preexisting fibers.

30 methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-

anticarboxylesterase deficiency; a disorder mediating the accumulation (e.g., storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (e.g., tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (e.g., Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin, methyldopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

Additionally, 33410 molecules may play an important role in the etiology of certain viral diseases, including but not limited to Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 33410 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, 33410 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

Additionally, 33410 may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, anorexia nervosa, cachexia, lipid disorders, and diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, e.g., inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, e.g., joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

As discussed, successful treatment of 33410 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, e.g., an agent identified using an assay described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 33410 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or

antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')<sub>2</sub> and Fab expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene  
 5 can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to  
 10 reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced  
 15 into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or  
 20 preventing a disease characterized by 33410 expression is through the use of aptamer molecules specific for 33410 protein. Aptamers are nucleic acid molecules having a tertiary structure that permits them to specifically bind to protein ligands (see, e.g., Osborne, et al. *Curr. Opin. Chem Biol.* 1997, 1(1): 5-9; and Patel, D.J. *Curr Opin Chem Biol* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently  
 25 introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which 33410 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in  
 30 instances whereby negative modulatory techniques are appropriate for the treatment of 33410 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 33410 protein or epitope for stimulating antibody production is harmful to the subject, it is possible

to generate an immune response against 33410 through the use of anti-idiotypic antibodies (see, for example, Herlyn, D. *Ann Med* 1999;31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A. *Cancer Treat Res* 1998;94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-  
 5 idiotypic antibodies, which should be specific to the 33410 protein. Vaccines directed to a disease characterized by 33410 expression may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells.  
 10 Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide  
 15 sequences encoding single-chain antibodies within the target cell population (see e.g., Marasco et al. (1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat or ameliorate 33410 disorders. A therapeutically effective dose refers to that amount of the  
 20 compound sufficient to result in amelioration of symptoms of the disorders.

Another example of determination of effective dose for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques. The compound which is able to modulate 33410 activity is  
 25 used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix that contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* 2:166-173.  
 30 Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al*

(1993) *Nature* 361:645-647. Through the use of isotope labeling, the “free” concentration of compound that modulates the expression or activity of 33410 can be readily monitored and used in calculations of  $IC_{50}$ .

Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual  $IC_{50}$ . A rudimentary example of such a “biosensor” is discussed in Kriz, D. *et al* (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 33410 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 33410 or agent that modulates one or more of the activities of 33410 protein activity associated with the cell. An agent that modulates 33410 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 33410 protein (e.g., a 33410 substrate or receptor), a 33410 antibody, a 33410 agonist or antagonist, a peptidomimetic of a 33410 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or 33410 activities. Examples of such stimulatory agents include active 33410 protein and a nucleic acid molecule encoding 33410. In another embodiment, the agent inhibits one or more 33410 activities. Examples of such inhibitory agents include antisense 33410 nucleic acid molecules, anti-33410 antibodies, and 33410 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 33410 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., up regulates or down regulates) 33410 expression or activity. In another embodiment, the method involves administering a 33410 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 33410 expression or activity.

Stimulation of 33410 activity is desirable in situations in which 33410 is abnormally down-regulated and/or in which increased 33410 activity is likely to have a beneficial effect.

For example, stimulation of 33410 activity is desirable in situations in which a 33410 is down-regulated and/or in which increased 33410 activity is likely to have a beneficial effect. Likewise, inhibition of 33410 activity is desirable in situations in which 33410 is abnormally upregulated and/or in which decreased 33410 activity is likely to have a  
 5 beneficial effect.

### Pharmacogenomics

The 33410 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 33410 activity (e.g., 33410 gene  
 10 expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 33410 associated disorders (e.g., neurological disorders and/or carcinomas) associated with aberrant or unwanted 33410 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign  
 15 compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 33410 molecule or 33410 modulator as well as tailoring the dosage  
 20 and/or therapeutic regimen of treatment with a 33410 molecule or 33410 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. et al. (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 and Linder, M.W. et al. (1997) *Clin. Chem.* 43(2):254-266. In general, two  
 25 types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase  
 30 deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high-resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach" can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a 33410 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling" can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 33410 molecule or 33410 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 33410 molecule or 33410 modulator, such as those identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 33410 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 33410 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., human cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a 33410 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 33410 gene expression, protein levels, or up-regulate 33410 activity, can be monitored in clinical trials of subjects exhibiting decreased 33410 gene expression, protein levels, or down-regulated 33410 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 33410 gene expression, protein levels, or down-regulate 33410 activity, can be monitored in clinical trials of subjects exhibiting increased 33410 gene expression, protein levels, or upregulated 33410 activity. In such clinical trials, the expression or activity of a 33410 gene, and preferably, other genes that have been implicated in, for example, a 33410-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

### **33410 Informatics**

The sequence of a 33410 molecule is provided in a variety of media to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains a 33410. Such a manufacture can provide a nucleotide or amino acid sequence, e.g., an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form. The sequence information can include, but is not limited to, 33410 full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequence, and the like. In a preferred embodiment, the manufacture is a machine-readable medium, e.g., a magnetic, optical, chemical or mechanical information storage device.



As used herein, "machine-readable media" refers to any medium that can be read and accessed directly by a machine, e.g., a digital computer or analogue computer. Non-limiting examples of a computer include a desktop PC, laptop, mainframe, server (e.g., a web server, network server, or server farm), handheld digital assistant, pager, mobile telephone, and the like. The computer can be stand-alone or connected to a communications network, e.g., a local area network (such as a VPN or intranet), a wide area network (e.g., an Extranet or the Internet), or a telephone network (e.g., a wireless, DSL, or ISDN network). Machine-readable media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM, ROM, EPROM, EEPROM, flash memory, and the like; and hybrids of these categories such as magnetic/optical storage media.

A variety of data storage structures are available to a skilled artisan for creating a machine-readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (e.g., text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

In a preferred embodiment, the sequence information is stored in a relational database (such as Sybase or Oracle). The database can have a first table for storing sequence (nucleic acid and/or amino acid sequence) information. The sequence information can be stored in one field (e.g., a first column) of a table row and an identifier for the sequence can be store in another field (e.g., a second column) of the table row. The database can have a second table, e.g., storing annotations. The second table can have a field for the sequence identifier, a field for a descriptor or annotation text (e.g., the descriptor can refer to a functionality of the sequence, a field for the initial position in the sequence to which the annotation refers, and a field for the ultimate position in the sequence to which the annotation refers. Non-limiting examples for annotation to nucleic acid sequences include

polymorphisms (e.g., SNP's) translational regulatory sites and splice junctions. Non-limiting examples for annotations to amino acid sequence include polypeptide domains, e.g., a domain described herein; active sites and other functional amino acids; and modification sites.

5 By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A  
10 search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif. The search can be a BLAST search or other routine sequence comparison, e.g., a search described herein.

Thus, in one aspect, the invention features a method of analyzing 33410, e.g., analyzing structure, function, or relatedness to one or more other nucleic acid or amino acid  
15 sequences. The method includes: providing a 33410 nucleic acid or amino acid sequence; comparing the 33410 sequence with a second sequence, e.g., one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database to thereby analyze 33410. The method can be performed in a machine, e.g., a computer, or manually by a skilled artisan.

20 The method can include evaluating the sequence identity between a 33410 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the Internet.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that  
25 the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

30 Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the

computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a sequence of a 33410 sequence that includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the following:

5 identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features a method of analyzing a sequence. The method includes: providing a 33410 sequence, or record, in machine-readable form;

10 comparing a second sequence to the 33410 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the 33410 sequence includes a sequence being compared. In a preferred embodiment the 33410 or second sequence is

15 stored on a first computer, e.g., at a first site and the comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the 33410 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of

20 a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention provides a machine-readable medium for holding instructions for performing a method for determining whether a subject has a 33410-

25 associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder, wherein the method comprises the steps of determining 33410 sequence information associated with the subject and based on the 33410 sequence information, determining whether the subject has a 33410-associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder and/or recommending a particular treatment for the

30 disease, disorder or pre-disease condition.

The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 33410-associated disease or disorder or a pre-disposition to a disease associated with a 33410 wherein the method comprises the steps of

determining 33410 sequence information associated with the subject, and based on the 33410 sequence information, determining whether the subject has a 33410-associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. In a preferred embodiment, the method further includes the step of receiving information, e.g., phenotypic or genotypic information, associated with the subject and/or acquiring from a network phenotypic information associated with the subject. The information can be stored in a database, e.g., a relational database. In another embodiment, the method further includes accessing the database, e.g., for records relating to other subjects, comparing the 33410 sequence of the subject to the 33410 sequences in the database to thereby determine whether the subject as a 33410-associated disease or disorder, or a pre-disposition for such.

The present invention also provides in a network, a method for determining whether a subject has a 33410 associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder associated with 33410, said method comprising the steps of receiving 33410 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 33410 and/or corresponding to a 33410-associated disease or disorder (e.g., a neurological disorder and/or carcinomas), and based on one or more of the phenotypic information, the 33410 information (e.g., sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 33410-associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a method for determining whether a subject has a 33410-associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder, said method comprising the steps of receiving information related to 33410 (e.g., sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 33410 and/or related to a 33410-associated disease or disorder, and based on one or more of the phenotypic information, the 33410 information, and the acquired information, determining whether the subject has a 33410-associated disease or disorder or a pre-disposition to a 33410-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

This invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

5

## EXAMPLES

### Example 1: Identification and Characterization of Human 33410 cDNA

The human 33410 sequence (Fig. 1; SEQ ID NO:1), which is approximately 4667 nucleotides long, including untranslated regions, contains a predicted methionine-initiated coding sequence of about 2508 nucleotides, including the termination codon (nucleotides indicated as “coding” of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence encodes a 835 amino acid protein (SEQ ID NO:2).

The location of the initiation and termination codons is indicated by the underline.

The human 33410 nucleic acid sequence is recited as follows:

15 GGCACGAGGCAACTTGGTCTGAATTCCAGGTCACCTAACCCTTGTCTCTTCTGTTTCCCAT  
TCCTTTCTGTCTGCCCCATCCAATTTCTTTGCCCTCTTCCACCTCTGTATTTTTCTGTCTG  
TCCGTCTGTCTGTATCCTGCCTCCCTGCCCTCTCGCTCCACCCCCCGCAGGTCGGGCTGC  
CTTCACCTTCTCCCACTTCTTCCCCTTCCCCACCCCGTGCCCCCTCCATGGAGAGGAACAG  
ACCCCTTCTCTGTCCAGTCTAACCAGGTCCCTCCCCAACCCCTCCTCCCTCCTTTCCCCC  
20 CGCCCCCTCCTCCCTCCTGGGGCGAGGGGGGCTCCCTCCCTCTCCCCCTTCTCTCTCTCT  
CCGAGGGGGGGGGTCCCAGGGAGGGAGGGGGGTCCCCGATCAGCATGTGGCTCCTGGCG  
CTGTGTCTGGTGGGGCTGGCGGGGGCTCAACGCGGGGAGGGGGTCCCGGCGGCGGCGCCCC  
GGGCGGCCCCGGCTGGGCCTCGGCAGCCTCGGCGAGGAGCGCTTCCCGGTGGTGAACACGG  
CCTACGGGCGAGTGCGCGGTGTGCGGCGGAGCTCAACAACGAGATCCTGGGCCCCGTCTGTG  
25 CAGTTCTTGGGCGTGCCCTACGCCACGCCGCCCTGGGCGCCCGCCGCTTCCAGCCGCTGA  
GGCGCCCGCCTCGTGGCCCGGCGTGCGCAACGCCACCACCCTGCCGCCCGCCTGCCCGCAGA  
ACCTGCACGGGGCGCTGCCCGCCATCATGTGCTGTGTGGTTACCGACAACCTTGGAGGCG  
GCCGCCACCTACGTGCAGAACAGAGCGAGGACTGCCTGTACCTCAACCTCTACGTGCCAC  
CGAGGACGGTCCGCTCACAAAAAACGTGACGAGGCGACGCTCAATCCGCCAGACACAGATA  
30 TCCGTGACCCTGGGAAGAAGCCTGTGATGCTGTTTCTCCATGGCGGCTCCTACATGGAGGGG  
ACCGGAAACATGTTTCGATGGCTCAGTCCTGGCTGCCTATGGCAACGTCATTGTAGCCACGCT  
CAACTACCGTCTTGGGGTGCTCGGTTTTCTCAGCACCGGGGACCAGGCTGCAAAAGGCAACT  
ATGGGCTCCTGGACCAGATCCAGGCCCTGCGCTGGCTCAGTGAAAACATCGCCCACTTTGGG  
GGCGACCCCGAGCGTATCACCATCTTTGGTTCCGGGGCAGGGGCCTCCTGCGTCAACCTTCT  
35 GATCCTCTCCCAACATTGAGAAGGGCTGTTCCAGAAGGCCATCGCCAGAGTGGCACCGCCA  
TTTCCAGCTGGTCTGTCAACTACCAGCCGCTCAAGTACACGCGGCTGCTGGCAGCCAAGGTG

003423-081001

GGCTGTGACCGAGAGGACAGTGCTGAAGCTGTGGAGTGTCTGCGCCGGAAGCCCTCCCGGGA  
 GCTGGTGGACCAGGACGTGCAGCCTGCCCGCTACCACATCGCCTTTGGGCCCCGTGGTGGATG  
 GCGACGTGGTCCCCGATGACCCTGAGATCCTCATGCAGCAGGGAGAATTCCTCAACTACGAC  
 ATGCTCATCGGCGTCAACCAGGGAGAGGGCCTCAAGTTCGTGGAGGACTCTGCAGAGAGCGA  
 5 GGACGGTGTGTCTGCCAGCGCCTTTGACTTCACTGTCTCCAACCTTTGTGGACAACCTGTATG  
 GCTACCCGGAAGGCAAGGATGTGCTTCGGGAGACCATCAAGTTTATGTACACAGACTGGGCC  
 GACCGGGACAATGGCGAAATGCGCCGCAAACCCCTGCTGGCGCTCTTTACTGACCACCAATG  
 GGTGGCACCCAGCTGTGGCCACTGCCAAGCTGCACGCCGACTACCAGTCTCCCGTCTACTTTT  
 ACACCTTCTACCACCACTGCCAGGCGGAGGGCCGGCCTGAGTGGGCAGATGCGGCGCACGGG  
 10 GATGAACTGCCCTATGTCTTTGGCGTGCCCATGGTGGGTGCCACCGACCTCTTCCCTGTAA  
 CTTCTCCAAGAATGACGTATGCTCAGTGCCGTGGTTCATGACCTACTGGACCAACTTCGCCA  
 AGACTGGGGACCCCAACCAGCCGGTGCCGCAGGATACCAAGTTCATCCACACCAAGCCCAAT  
 CGCTTCGAGGAGGTGGTGTGGAGCAAATTCAACAGCAAGGAGAAGCAGTATCTGCACATAGG  
 CCTGAAGCCACGCGTGCGTGACAACCTACCGCGCCAACAAGGTGGCCTTCTGGCTGGAGCTCG  
 15 TGCCCCACCTGCACAACCTGCACACGGAGCTCTTACCACCACCACGCGCCTGCCTCCCTAC  
 GCCACGCGCTGGCCGCTCGTCCCCCGCTGGCGCCCCGGGCACACGCCGGCCCCCGCCGCC  
 TGCCACCCTGCCTCCCGAGCCCGAGCCCGAGCCCGGCCCAAGGGCCTATGACCGCTTCCCCG  
 GGGACTCACGGGACTACTCCACGGAGCTGAGCGTCACCGTGGCCGTGGGTGCCTCCCTCCTC  
 TTCTCAACATCCTGGCCTTTGCTGCCCTCTACTACAAGCGGGACCGGCGGCAGGAGCTGCG  
 20 GTGCAGGCGGCTTAGCCACCTGGCGGCTCAGGCTCTGGCGTGCCTGGTGGGGCCCCCTGC  
 TCCCCGCGCGGGCCGTGAGCTGCCACCAGAGGAGGAGCTGGTGTCACTGCAGCTGAAGCGG  
 GGTGGTGGCGTCGGGGCGGACCTGCCGAGGCTCTGCGCCCTGCCTGCCCCCGGCGACTACAC  
 CCTGGCCCTGCGCCGGGCACCGGACGATGTGCCTCTCTTGGCCCCCGGGGCCCTGACCTGC  
 TGCCCAGTGGCCTGGGGCCACCGCCACCCCCACCGCCCCCTCCCTTCATCCCTTCGGGCCC  
 25 TTCCCCCGCCCCCTCCACCGCCACCAGCCACAACAACACGCTACCCACCCCCACTCCAC  
 CACTCGGGTATAGGGGGTGGGTGGGGAGGCCCTCCTCCCCGGCCCTCCCTGGCCCCGGCCACT  
 CCGAAGGCAGGGAGGAGGACTTGGCAACTGGCTTTTCTCCTGTGGAGTCGTACACGCCATC  
 CAGCAGCGCTAAGGTGGACATGGGATTCTCCCTGCGATGCGTGTCTTTCCACGCAGAGAA  
 GCCCCAGTCTCTTCTCTGGATCTGGGCCTTTGAACAACCTGGGGGGCGTTTTCTCCCCCAT  
 30 TGGGACACCAGTCTTCGGTGTGTGGAATGTGGTATTTTCCCGCGTGGAGGTGTGCTTTCTCA  
 CAACGGGGTGTGTTTTCCCATGTGCAGGGTGAGGTTTTTTTTTGGCACCCCTGGACACATGTT  
 GGCCCCCTCAAAGAATTTCTGTGGGGATTTGTACCCAGAAATCCTGTTCCCCCATCCCTTCT  
 CCCACCTCCTCCCCCTCTCCCTCCCCCTGGAGACCCTGGAAGTGGTGTGTTACATACAGTGA  
 CCCTTGGCCACCAGACCACAGAGGATGGAGCCTGGGAAGCAGCGAGGAAATCACAGCCCCCT  
 35 CGCCCCCTGCCTCCCTTGCCCCCTACCCCGGCGAAGCATGTTCCCCCGACGCCCCCTTGGA  
 CAAGTCAGATGAAGCACGTTCTGCCGGGGAGGCCCTCACCTTCCAGAGAGGACAGACACAGA  
 TTTCTCTGCTGGGGGAGGGAGGAGTCCACGCATCCTGATGCTGCCTGGAAGCTTATTTTCCCG  
 TGGCCAGGACGCATTTCTCTGAGTGGAACAGGTTCTTGCATGTGGATGTGTGTTTCCCG

GCAGACGGCCCCCTCTCTTCCCAGCACTTCCCTGCCTCCCCCAGGCCTCAGGCCCAGCACCCA  
 GTTCCTCCTCACATGGCAGGTGAGCACAGACTTCTAGTTGGCAGGAGCTGAGGAGGGTGAAC  
 AAACCCCGAGGGAGGCCCGGCCCTTGCTCCCGAGTTGGGGGGAGGGGGTGTGGCAACGTGCC  
 CCCCAGAGGCCACGCATGTTTGACCAAAGCCCTCATTGTGGTCCGAGGACAGCCTTTTCC  
 5 CCAGGCCTCAGAGCATTGCTCATCCGTGCCAACTGGGTAGGTGGATTGTAGCGGAAAGACT  
 CCCAAAATGTGCCAAGAATTTCCCAGTCCCAGGCAGGGCAGGGGAAACTAAGGGCAAGCAGG  
 ATACAGGGCGAGGGATGTGGCAGGTGAGGGGGCTCCCGCCTGTGCCCCCTTCTCCTCACCATG  
 TCTCCCCCACCCTGCCTCAGTTCTCCGTCCCCCTTCATCTCCGTCCCCCTCTTTGAAGCTGT  
 CCCCATCTCAGTGTGAGACCAGCCTTCTCCTCATCTGACCACCCTCCTCTGACCGACGCCCC  
 10 CTCCTTGTCTGAAAGAAAGGAGCCTTGAATGGTGGAGGGAGGCAGTGGGGAGAAAGGTCTCA  
 CCGGACAGGTTGGGAGAATGAGGTGAGCGGTGCTGGGGAACAGATGGAGGGGGCAGTGGGGA  
 CAGGGCTTGGGCAGACACCAGCAGGAATAATTTGAAATGTGTGAGGTGACTCCCCGGAGGGC  
 CTTGGGCTTGGGCATTTGGGAAAAGAATGATGTCTGGAAGGGCTTAAGGGACACAGTGGACG  
 AGGGGAGAGTCTCATCTGCTGGCATTTTGTGGGGTGTAGTGCCAACTTGAATAGGGGCT  
 15 GGGGTGCTGTCTTCCACTGACACCCAAATCCAGAATCCCTGGTCTTGAGTCCCAGAACTTTG  
 CCTCTTGACTGTCCCTC (SEQ ID NO:1).

The coding sequence encodes a 835 amino acid protein (SEQ ID NO:2) and has the following amino acid sequence:

MWLLALCLVGLAGAQRGGGGPGGGAPGGPGLGLGSLGEERFPVNTAYGRVRGVRRELNNEI  
 20 LGPVVQFLGVPYATPPLGARRFQPPEAPASWPGVRNATTLPPACPQNLHGALPAIMLPVWFT  
 DNLEAAATYVQNQSEDCLYLNLYVPTEDGPLTKKRDEATLNPPDTDIRDPGKKPVMLFLHGG  
 SYMEGTGNMFDGSVLAAYGNVIVATLNYRLGVLGFLSTGDQAAKGNYGLLDQIQALRWLSEN  
 IAHFGGDPERITIFGSGAGASCVNLLILSHHSEGLFQKAIASQGTAISSWSVNYQPLKYTRL  
 LAAKVGCDREDSAEAVECLRRKPSRELVDQDVQPARYHIAFGPVVDGDVVPDDPEILMQQGE  
 25 FLNYDMLIGVNQGEGLKFVEDSAESEDGVSASAFDFTVSNFVDNLYGYPEGKDVLRETIKFM  
 YTDWADRDNEMRRKTLLALFTDHQWVAPAVATAKLHADYQSPVYFYTFYHHCQAEGRPWA  
 DAAHGDELPHYVFGVPMVGATDLFPCNFSKNDVMLS AVVM TYWTNFAKTGDPNQVPVQDTKFI  
 HTKPNRFEEVVWSKFNSKEKQYLHIGLKPRVRDNYRANKVAFWLELVPHLHNLHTELFTTTT  
 RLPPYATRWP RP RP PAGAPGTRRP PPPATLPPEPEPEPGPRAYDRFPGDSRDYSTELSVTVAV  
 30 GASLLFLNILAFAALYYKRDRRQELRCRRLSPPGSGSGVPGGGPLLPAAGRELPPPEELVS  
 LQLKRGGGVGADPAEALRPACPPDYTLALRRAPDDVPLLAPGALTLLPSGLGPPPPPPPSL  
 HPFGFPFPFPPTATSHNNTLPHPHSTTRV (SEQ ID NO:2).

## Example 2: Tissue Distribution of 33410 mRNA by Large-Scale Tissue-Specific Library

### 35 Sequencing and by Northern Blot Hybridization

Endogenous human 33410 gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology. Briefly,

TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) that has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of Taq polymerase digests the labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle where fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a way of quantitating the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe that has been labeled with a different fluorophore on the 5' end (typically VIC) and is directed toward, for example, a housekeeping gene such as GAPDH.

To determine the level of 33410 in various human tissues a primer/probe set was designed using Primer Express (Perkin-Elmer) software and primary cDNA sequence information. Total RNA was prepared from a series of human tissues using an RNeasy kit from Qiagen. First strand cDNA was prepared from one  $\mu$ g total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng total RNA was used per TaqMan reaction.

Tissues tested included the human tissues shown in the Table below, including ovary, kidney, spleen, lung, liver, and colon, among others. Expression was found primarily in normal brain cortex, with expression to a lesser extent in normal artery, normal heart, normal kidney, normal spinal cord, normal brain hypothalamus, and normal ovary (Table below). Expression of 33410 RNA in a human liver panel using TaqMan analysis with the following tissues were performed: heart, kidney, skeletal muscle, and several samples of normal and diseased liver. Elevated expression was detected in heart tissue.



Table 1: Expression Data

Tissue Type	Relative Expression
Artery normal	2.4894
Aorta diseased	0
Vein normal	0
Coronary SMC	0
HUVEC	12.9581
Hemangioma	0.94
Heart normal	0.398
Heart CHF	0
Kidney	0.2608
Skeletal Muscle	0
Adipose normal	0
Pancreas	0
primary osteoblasts	0
Osteoclasts (diff)	0
Skin normal	0
Spinal cord normal	1.3621
Brain Cortex normal	68.8691
Brain Hypothalamus normal	9.3553
Nerve	0
DRG (Dorsal Root Ganglion)	0
Breast normal	0
Breast tumor	0
Ovary normal	1.4957
Ovary tumor	0
Prostate normal	0
Prostate tumor	0
Salivary glands	0
Colon normal	0
Colon tumor	0
Lung normal	0
Lung tumor	0
Lung COPD	0
Colon IBD	0
Liver normal	0
Liver fibrosis	0
Spleen normal	0
Tonsil normal	0
Lymph node normal	0
Small intestine normal	0
Macrophages	0
Synovium	0
BM-MNC	0
Activated PBMC	0

Neutrophils	0
Megakaryocytes	0
Erythroid	0
positive control	11.3592

Table 1 depicts the expression of 33410 RNA in a panel of normal and tumor human tissues detecting using TaqMan analysis. Elevated expression was detected in normal artery, HUVEC, hemangioma, normal heart, kidney, normal spinal cord, normal brain cortex, brain hypothalamus and normal ovary.

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 33410 cDNA (SEQ ID NO:1) can be used. The DNA was radioactively labeled with <sup>32</sup>P-dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

#### Example 3: Recombinant Expression of 33410 in Bacterial Cells

In this example, 33410 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 33410 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-33410 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

#### Example 4: Expression of Recombinant 33410 Protein in COS Cells

To express the 33410 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 33410 protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag

fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 33410 DNA sequence is amplified by PCR using two  
 5 primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 33410 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 33410 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested  
 10 with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably, the two restriction sites chosen are different so that the 33410 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 $\alpha$ , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on  
 15 ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 33410-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for  
 20 transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 33410 polypeptide is detected by radiolabeling ( $^{35}\text{S}$ -methionine or  $^{35}\text{S}$ -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D.  
 25 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with  $^{35}\text{S}$ -methionine (or  $^{35}\text{S}$ -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated  
 30 with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 33410 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting

plasmid is transfected into COS cells in the manner described above, and the expression of the 33410 polypeptide is detected by radiolabeling and immunoprecipitation using a 33410 specific monoclonal antibody.

5 Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

10448-081001